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Isoperoxidase analysis on *Thelypteris ciliata* (Wall. ex Benth.) Holttum (Thelypteridaceae)

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ABSTRACT

Objective: To assess the genetic variation between different populations of *Thelypteris ciliata* (Wall. ex Benth.) Holttum *(T. ciliata)* collected from different localities on Tirunelveli Hills (Kakachi, Kothayar Upper Dam) India, using isoperoxidase. **Methods:** The matured sterile and fertile fronds were harvested and served as explants for the isoperxidase analysis. The isoperoxidase isolation, separation and staining were followed by Smila et al. method. **Results:** A total of six bands in six different positions with five active regions were observed in the isoperoxidase enzyme system of *T. ciliata*. **Conclusions:** The present study confirms the intra and inters- population's genetical difference of *T. ciliata*.

1. Introduction

Plants are the major sources of medicines. Members of all the groups of plants are medicinally important. When compared to other groups of plants, ferns and fern allies are generally considered as poor source of medicinal plants due to the difficulties in collection and identification. There are several reports, from ancient time, about the medicinal importance of ferns and fern allies. For example, the species of Asplenium usually called spleenworts, are used to cure spleen diseases. Among the ferns, the genus Thelypteris is a large one with several hundred species present throughout the world, particularly in tropical countries. About twenty five thelypteroid ferns are present in south India [1]. The medicinal importance of several south Indian thelypteroid ferns has been explored through studies on ethnobotany, phytochemistry and antimicrobial activity. The roots of Thelypteris palustris are used to treat women complaints in America [2]. Antibacterial activity has been reported in several thelypteroid ferns such as Christella parasitica [3, 4]. Detailed studies on morphology, biochemistry and bioactivities on epidermal glands of six thelypteroid ferns from south India have been carried out by Paulraj [5] and Paulraj *et al* [4]. Bioactive compounds such as, flavonoid glycosides have been reported from Christella parasitica (L.) Lev. from the Western Ghats, South India [6]. New cytotoxic flavonoids have been isolated from Thelypteris torresiana [7]. Drimanes and other terpenoids are present in the fern Thelypteris hispidula (Decne.) Reed[8]. *Thelypteris ciliata* (*T. ciliata*) is also a common thelypteroid species commonly present in tropical countries, including India. It is a complex species with several morphotypes and ecotypes. It is a small or large herb growing along shaded streams at medium or high altitude [1]. The fronds are monomorphic simply pinnate without major difference between fertile and sterile fronds. The young sori are pink in colour and the pink colour is due to the pink coloured annulus of the sporangia. Generally all the thelypteroid species are with monomorphic fronds. Distinct dimorphic fronds are commonly present in ferns from the primitive family like, Ophioglossaceae (Ophioglossum, Botrychium) to advanced family Polypodiaceae (Leptochilus, Pyrrosia etc). It is expected that there may be some degree of difference in phytochemistry and bioactivity between the fertile and sterile fronds. Kale and Upadhye [9] have studied the proteins in the dimorphic leaves of three medicinal ferns (Drynaria quercifolia, Ceratopteris thalictroides, Bolbitis appendiculata) of the Western Ghats, south India. The present study has been aimed to know the isozymic

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variation between the fertile and sterile monomorphic fronds of *T. ciliata*. Usually, under environmental stress conditions, more tolerable plant species produces variety of chemical substances to cope with the environment and such species are usually with several useful bioactive compounds. It is to be noted that *T. ciliata* is a tolerable species in which the young sori are pink in colour due to the presence of pink coloured annulus of the sporangium [1]. The presence of anthocyanin in the sporangial wall itself shows that the sporangial wall give not only the mechanical protection but also physiological protection against UV radiation for the developing spores, by the absorption of UV radiation by the anthocyanin pigments present in the annulus of the sporangium. Thus it is reasonable to expect the phytochemical and molecular differences between the fertile and sterile fronds of *T. ciliata*.

The modern molecular approaches have been adopted to classify the pteridophytes, in order to distinguish the taxon. Isoenzyme marker is the oldest, simple and inexpensive method of obtaining genetic information. Isoenzyme has been proven to be reliable genetic marker in plant systematic studies due to its consistency in their expression, irrespective of environmental factors. In addition, it allows quantification of the genetical homology and distance within and between species[10]. Poly Acrylamide Gel Electrophoresis is a versatile biochemical technique to detect genetic variation. In recent years there has been an explosion in the number of different types of genetic markers available, moreover the number of statistical have been developed for analyses of molecular data, in most cases, the new DNA based markers provide the same type of information as allozymes, but allow for clear resolution of genetic differences. This method has been used for the identification of some medicinally and horticulturally important plants, to study the phylogenetic affinities in medicinal and commercial crops [11-16]. Isozymes are used not only as markers of inter-specific and intra-specific taxa, but also they may be used as markers of different developmental stages of the same plant. It is of no doubt that there will be much of metabolic differences between the fertile and sterile fronds of ferns. It is necessary to understand the phytochmeical and molecular differences among the fertile and sterile fronds of the medicinal ferns for successful utilization and identification. In the present study it has been aimed to assess the variation in the expression of isoperoxidase isozyme in fertile and sterile fronds of *T. ciliata* in different populations of *T. ciliata* collected from different localities on Tirunelveli Hills (Kakachi, Kothayar Upper Dam) India.

2. Materials and methods

Plants of *T. ciliata* (Wall. ex Benth.) Holttum collected from natural habitats of different localities on Tirunelveli Hills (Kakachi, Kothayar Upper Dam) and were established in the green house attached to St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India. Croziers were harvested from the mother plant and served as explants for the isoperoxidase analysis. The matured sterile and fertile fronds were harvested and served as explants for the isoperxidase analysis. The isoperoxidase isolation, separation and staining were followed by Smila *et al*. method [17]. The isoperoxidase profiles were visualized and documented in Vilber Lubermat, Germany, and analysis of similarity and variation between the selected species were carried out using Biogene software, Germany.

3. Results

A total of six bands in six different positions with five active regions were observed in the isoperoxidase enzyme system of *T. ciliata*. No band was expressed in zone one and two. One band PRX31 (0.280) was observed in zone three, and it was present only in fertile population collected from Kothayar-Upper Dam. Zone four also showed only one band (PRX41) with the R_f value (0.384) in sterile plants of population collected from Kothayar- Upper Dam. Zone five showed a single band (PRX51) with the Rf value 0.405 in fertile plants of the same population. Zone six showed two bands in two different positions. PRX61 (0.596) was expressed only in sterile plants of population collected from Kothayar-Upper Dam. PRX62 (0.580) showed the restricted presence in fertile plants of population collected from Kothayar- Upper Dam. Region seven expressed with a single band with the Rf value 0.698 in population collected from Kakachi stream (Table 1; Figure 1).

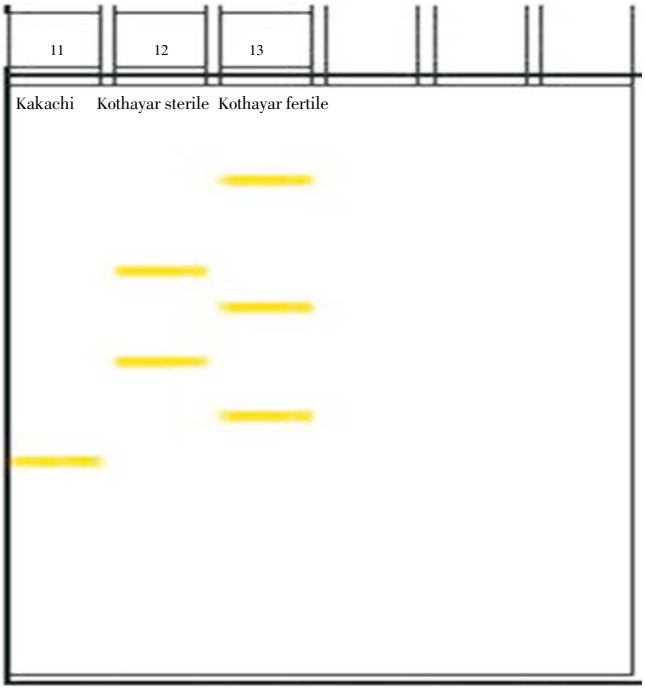

Figure 1. Isoperoxidase profile of *T. ciliate*

Table 1
Isozymic Banding Pattern of *T. ciliata*.

MW – R_f	Isoform	Kakachi – Fertile	Kothayar Upper Dam – Sterile	Kothayar Upper Dam Fertile
0.280	PRX31	–	–	+
0.384	PRX41	–	+	–
0.405	PRX51	–	–	+
0.536	PRX61	–	+	–
0.580	PRX62	–	–	+
0.698	PRX71	+	–	–

4. Discussion

Based on the present isozymic analysis it is concluded that the different poplulations of *T. ciliata* show genetical differences. Thus the population from Kakachi shows the presence of only one band in contrast to the presence of five different bands (2 in sterile, 3 in fertile) in the plants of population from Kothayar-Upper Dam. It is important to note the isozymic difference between the fertile and sterile plants of the same population. Plants of the above two populations also differ in size. The plants in the population from Kakachi stream are larger with about 50 cm long fronds in contrast to the plants in the population from Kothayar-Upper Dam with about 20-30 cm long fronds. In general the species of Trigonospora from south India are highly variable morphologically, cytologically and ecologically. *T. ciliata* with larger fronds is tetraploid [18]. Molecular studies on several populations from different localities of south India will be more useful to understand the interspecific and intraspecific relationships of Trigonospora from south India.

Conflict of interest statement

We declare that we have no conflict of interest.

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