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## Isozymic variation among three filmy ferns belonging to different morphological forms growing in different ecological niche

M.Johnson<sup>\*\*</sup>, S.Dominic Rajkumar, V.Irudayaraj

Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, (INDIA)

<sup>#</sup>Department of Botany, St. Andrew's College, Gorakhpur - 273 001, (INDIA)

E-mail : ptcjohnson@gmail.com

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### ABSTRACT

The present study was aimed to identify isozymic variation among three filmy ferns viz., *Trichomanes obscurum* Bl., *Trichomanes proliferum* Bl. and *Trichomanes plicatum* (V.D.B) Beddome belonging to different morphological forms growing in different ecological niche. The standard technique of vertical poly acrylamide gel electrophoresis was employed to demonstrate the banding profile and the gels were stained for isoperoxidase enzyme system. A total of sixteen bands were obtained in this enzyme system. Bands were observed in between zone four to nine and other zones were failed to express in this isoperoxidase system. The present biochemical study is in agreement with the above morphological and cytological evolution of filmy ferns. Thus the present study clearly shows the genetic distinction of all the three species and supporting the multigeneric system of classification in filmy ferns. © 2010 Trade Science Inc. - INDIA

### KEYWORDS

*Trichomanes obscurum*;  
*Trichomanes proliferum*;  
*Trichomanes plicatum*;  
 Isozymes.

### INTRODUCTION

The filmy fern genus *Trichomanes* is represented by six species in south India<sup>[1]</sup>. Among the six species, three species *Trichomanes obscurum* Bl., *Trichomanes proliferum* Bl. and *Trichomanes plicatum* (V.D.B) Beddome are common, remaining three species *Trichomanes schmidianum* Zenker ex Taschn, *Trichomanes saxifragoides* Presl and *Trichomanes intramarginale* Hook. & Grev. are rare. Common species are large complexes with presence

of several morphotypes and cytotypes. One major problem in the generic delimitation of filmy fern is the bigeneric system or multigeneric system of classification. Such problem has been analysed by morphotaxonomy, cytotaxonomy and to some extent by chemotaxonomy<sup>[2]</sup>. Majority of the studies are more or less in accordance with the multigeneric system of classification. But so far there is no strong evidence to prove which type of classification is correct. Electrophoresis joined with zymogram technique has been the tool of choice for studies of heritable variation by ge-

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**TABLE 1 : Isoperoxidase banding pattern of some species of *Trichomanes***

MW-Rf values	Isoperoxidase Profile	<i>Trichomanes obscurum</i>	<i>Trichomanes proliferum</i> (Kakachi)	<i>Trichomanes proliferum</i> (Manjolai)	<i>Trichomanes plicatum</i>
0.354	PRX4 <sup>1</sup>			+	
0.446	PRX5 <sup>1</sup>				+
0.540	PRX6 <sup>1</sup>				+
0.556	PRX6 <sup>2</sup>			+	
0.605	PRX7 <sup>1</sup>		+	+	+
0.616	PRX7 <sup>2</sup>	+			
0.635	PRX7 <sup>3</sup>		+	+	
0.663	PRX7 <sup>4</sup>	+			+
0.698	PRX7 <sup>5</sup>	+			
0.755	PRX8 <sup>1</sup>				+
0.812	PRX9 <sup>1</sup>				+
0.854	PRX9 <sup>2</sup>				+

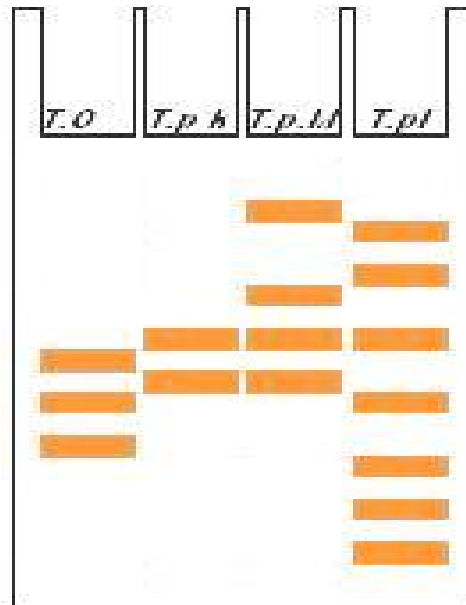
neticists, systematists and population biologist<sup>[3]</sup>. Isozymes are powerful tool for gene variability within and between the population of plants and animals<sup>[4-8]</sup>. The three common filmy ferns are put under either a single genus *Trichomanes* or three different genera such as *Scenodesmium*, *Gonocormos* and *Crepidomanes*. In order to know the genetical relationships and to test the generic classification, the above three species were subjected to isoperoxidase analysis.

### MATERIALS AND METHODS

Plants of *Trichomanes obscurum* Bl., *Trichomanes proliferum* Bl. and *Trichomanes plicatum* (V.D.B) Beddome were collected from the natural habitats and established in the green house attached to Kodaikanal Botanic Garden, Kodaikannal, Tamil Nadu and India. Croziers were harvested from the mother plants and served as explants for the isoperoxidase analysis. The isoperoxidase isolation, separation and staining was followed by Smila et al., method<sup>[9]</sup>. The isoperoxidase profiles were visualized and documented in Vilber Lobermat (Germany) and similarity and variation between the selected species were carried out by Biogene software (Germany).

### RESULTS AND DISCUSSION

A total of sixteen bands were obtained in this en-



zyme system. Bands were observed in between zone four to nine and other zones were failed to express in this isoperoxidase system. Zone four showed a single band (PRX4<sup>1</sup> – 0.354) with the specific presence in *T. proliferum* collected from Kothayar. PRX5<sup>1</sup> – 0.446 and PRX6<sup>1</sup> – 0.540 were restricted to *T. plicatum*. PRX6<sup>2</sup> unique to *T. proliferum* collected from Kothayar. PRX7<sup>1</sup> was commonly shared by the selected three filmy ferns. PRX7<sup>2</sup> was only present in *T. obscurum*. PRX7<sup>3</sup> was shared by two populations of *T. proliferum*. PRX7<sup>4</sup> was showed the presence jointly in *T. obscurum* and *T. plicatum*. PRX7<sup>5</sup> was expressed only in *T. obscurum*. Zone eight showed a single band and it is specific to *T. plicatum* and Zone nine showed two bands in two different positions and present only in *T. plicatum*.

The present study on evolutionary relationships among three distinct filmy ferns shows high degree of genetic diversity in *T. plicatum* with seven bands and low degree of genetical diversity in one population of *T. proliferum* collected from Kakachi, Tirunelveli Hills. Morphologically the distinct species *T. obscurum* shows only three bands. *T. obscurum* is the larger species among filmy ferns with large frond (50 × 12cm), typically tubular involucre and tripinnatifid lamina. This species has distinct chromosome number  $n = 33$ <sup>[10,11]</sup>. *T. plicatum* (*Crepidomanes plicatum*) is a small (8 × 3.5cm) sized plants with tripinnatifid lamina and half way free flattened involucre, cytologically it is variable with regular 36 bivalents in spore

mother cells or with irregular meiosis<sup>[12]</sup>. *T. proliferum* is small (7.5 × 1.5cm) sized plants with pinnate or flabellately divided lamina with obconical winged involucre and it is highly variable in morphology and cytology. Of the above three species, *T. plicatum* has numerous false veins on either side of the true vein.

The present biochemical study is in agreement with the above morphological and cytological evolution of filmy ferns. Thus, the primitive largest filmy fern with distinct chromosome number (n = 33) has only three bands. The morphologically advanced species (*T. proliferum*) with small sized lamina and low degree of cutting of pinnae shows two bands in one population (kakachi) and four bands in another population (Manjolai). In contrast, the morphologically and cytological intermediate species *T. plicatum* shows the presence of maximum number of bands<sup>[7]</sup>. *T. plicatum* is also highly variable in morphology particularly in size and dissection of lamina. Presence of maximum degree of genetic diversity in *T. plicatum* may be due to high stability with variety of environment. Thus, *T. plicatum* grows either as epiphyte or lithophyte between 100 to 1850m, in contrast to the primitive species *T. obscurum* which grows commonly as terrestrial plants between 1050 to 1300m and the advanced species *T. proliferum* with low degree of genetic diversity grows as large colonies along streams or fully shaded rocks between 500 to 1300m<sup>[1]</sup>. Out of sixteen bands observed in isoperoxidase enzyme system among three species, none of the band is shared by all the three species but one band is shared by *T. plicatum* and *T. obscurum*. Thus the present study clearly shows the genetic distinction of all the three species and supporting the multigeneric system of classification in filmy ferns. Such genetic diversity may be due to the habitat variation.

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