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Isozymic studies on selected species of *Pteris* from India

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ABSTRACT

The present study was aimed to illustrate the isoperoxidase banding profile for some species of *Pteris* from India. 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. In this enzyme system, a total of thirty eight bands were scored in thirty one different positions with eight active zones. The present study revealed the biochemical positions of the *Pteris* complex. The similarity index and variations between these five species are reported with reference to isoperoxidase profiles.

KEYWORDS

Isozymes;
Iso-peroxidases;
Pteis argyraea;
Pteris otaria;
Pteris vitata;
Pteris biaurita;
Pteris confuse.

INTRODUCTION

Ferns and fern allies, collectively called Pteridophytes, are the first successful group of land plants without flowers and seeds. India is one of the important megabiodiversity countries with the presence of about 1200 species of Pteridophytes which are growing on the high altitude mountains like the Western Ghats and Himalayas. *Pteris* is a highly evolved tropical fern genus with the presence of several species complexes like *Pteris quadriaurita*, *P. cretica* and *P. vittata* with several morphotypes and cytotypes. Walker[1] has observed introgression resulting in the formation of hybrid swarm in nature. Majority of the species are sexual diploids or tetraploids and some species are diploid or triploid apogamous. With the chance for easy hybridization, several morphotypes and cytotpyes occur in nature. Ferns in general, *Pteris* species in particular, play an important role in phytoremediation throughout the world. The Chinese Brake fern *Pteris vittata* have been studied extensively for its potentiality in hyperaccumulation of Arsenic[2]. In a recent study the spores of related species *Pteris confusa* T. G. Walker and *P. argyraea* T. Moore shows reverse response in heavy metal tolerance during germination[3]. There are 15 species of *Pteris* in south India[4]. General taxonomical, cytological and phytochemical studies on south Indian *Pteris* have been done[4-6] and the results show the high degree of morphological, cytological and chemical diversity of south Indian species of *Pteris*. Isozyme analysis reveals the systematic positions of several taxa with inexpensive technique[7,8]. In 1960s, biochemical methods based on seed, leaves protein or enzyme electrophoresis were introduced, which proved particularly useful in analysis of genetic diversity as they reveal differences between seed storage protein encoded by different

alleles at one (allozymes) or more gene loci (isozymes). Use of biochemical methods eliminates the environmental influence. These methods are being used as complementary strategies to traditional approaches for assessment of genetic diversity. The analysis can be performed at any growth stage using any plant part and it requires only small amounts of materials. Following the advances in molecular biology in the last decade a variety of different methods have been developed for analysis of genetic diversity. In order to know the genetical diversity among the species of *Pteris* from South India, isozyme analysis has been done in some common species of *Pteris* particularly from *Pteris quadriaurita* complex along with another unrelated species *Pteris vittata*.

MATERIALS AND METHODS

Pteis argyraea, Pteris otaria, Pteris vittata, Pteris biaurita and *Pteris confus*a were collected from different localities of the Western Ghats, South India and established in the green house at Centre for Biodiversity and Biotechnololgy, St. Xavier's College, Palayamkottai, Tamil Nadu, India. The croziers were harvested from mother plants and used as the experimental materials for the enzyme isolation. 500 to 1000 mg of freshly harvested young croziers were taken and homogenized with 3.5 ml of ice-cold homogenizing buffer (0.1M phosphate buffer (pH 7.0)) and centrifuged at 12,000 rpm for 10 min. The supernatant was subjected to electrophoresis (PAGE) as described by Anbalagan[9]. For the detection of isozymes on the gels, the staining solution, were prepared as per Smila et al., method[10]. After the electrophoresis, the gels were incubated in the staining solution for few minutes under dark condition till the clear bands appeared. The gels were fixed with 7% acetic acid solution for 30 min, washed with distilled water and banding patterns were documented using the Vilber Loubermat gel documentation system (Germany) and the similarity index and variations were calculated using the Biogene software (Germany).

RESULTS AND DISCUSSION

Isoperoxidase enzyme system showed the multiple zones[8] of activity with different banding profiles. Zone one and two failed to express the banding profile for the selected ferns in this enzyme system. Zone 3 contained two bands in two different positions. Band PRX 3^1 (0.275) showed its presence in *P. otaria* and *P. confusa*. PRX 3^2 (0.284) was shared by *P. argyraea* and *P. biaurita* collected from Kothayar (2 x). Zone 4 showed three bands in three different positions. PRX 4^1 (0.304) showed its unique presence in *P. otaria* only. PRX 4^2 (0.375) was unique to *P.argyraea* and PRX 4^3 (0.400) was restricted with diploid *P. biaurita* collected from Kothayar. Thus the zone 4 played a key role in characterization of *P. otaria, P. argyraea* and *P. biaurita.* Zone 5 contained three bands in three different positions. PRX 5^1 (0.422) was unique to triploid apogamous *P. biaurita* collected from Kodaikanal botanic garden. PRX 5^2 (0.481) was restricted to *P. otaria* and PRX 5^3 (0.491) for *P. argyraea*. Thus the zone 5 played a key role in the characterization of triploid apogamous *P. biaurita* collected from Kodaikanal botanic garden. Zone 6 showed six bands with six different position of expression. PRX 6^1 (0.534) was shared by *P. biaurita* (Kothayar populations) and *P. argyrae*a. PRX 6^2 (0.555) was commonly present in *P. otaria* and *P. biaurita* (Kothayar). PRX 6^3 (0.567) was restricted to *P.confusa*, PRX 6^4 (0.574) was unique to *P. argyraea*, PRX 6^5 (0.579) was present only in *P. vittata* and PRX 6^6 (0.588) was exclusive to *P. biaurita* (Kothayar). Zone 7 also expressed six bands in six different banding patterns. PRX $7^{1\,\&\,6}$ (0.601 and 0.683) was unique to *P.viattata,* PRX 7^3 (0.629) was restricted to *P. argyraea*, PRX 7^4 (0.634) showed its presence in *P. biaurita* (Kodaikanal botanic garden populations), PRX 7^2 (0.613) was shared by *P. confusa* and *P. vittata*. Zone 8 recorded six bands with different banding positions. PRX $8^{1\text{ and }3}$ (0.710 and 0.748) were unique to *P. vittata*. PRX $8^{4\text{ and }6}$ (0.760 and 0.779) were restricted with *P. argyraea*. PRX 8^5 (0.768) to *P. biaurita* Kodaikanal botanic garden population. PRX 8^2 (0.723) showed the common presence in *P. argyraea* and *P. vittata*. Zone 9 showed four bands in four different positions in the enzyme system. PRX $9^{1\text{ and }4}$ (0.809 and 0.890) were unique to *P. biaurita* from Kodaikannal Botanic Garden. PRX 9^2 (0.818) was restricted to *P. confusa* and PRX 9^3 (0.845) was shared by *P. biaurita* (KBG) and *P. argyraea*. Zone 10 showed a single band PRX 10^1 (0.926) and it is restricted to *P. biaurita* from KBG.

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

Rf values	Isoperoxidase profile	*P. otaria*	*P. confusa*	*P. biaurita* (KBG)	*P. biaurita* (Kothai)	*P. argyraea*	*P. vittata*
0.275	PRX3[1]	+	+				
0.284	PRX3[2]				+	+	
0.304	PRX4[1]		+				
0.375	PRX4[2]					+	
0.400	PRX4[3]				+		
0.422	PRX5[1]			+			
0.481	PRX5[2]	+					
0.491	PRX5[3]					+	
0.534	PRX6[1]				+	+	
0.555	PRX6[2]	+			+		
0.567	PRX6[3]		+				
0.574	PRX6[4]					+	
0.579	PRX6[5]						+
0.588	PRX6[6]			+			
0.601	PRX7[1]						+
0.613	PRX7[2]		+				+
0.629	PRX7[3]					+	
0.634	PRX7[4]			+			
0.650	PRX7[5]		+				
0.683	PRX7[6]						+
0.710	PRX8[1]						+
0.723	PRX8[2]					+	+
0.748	PRX8[3]						+
0.760	PRX8[4]					+	
0.768	PRX8[5]			+			
0.779	PRX8[6]					+	
0.809	PRX9[1]			+			
0.818	PRX9[2]		+				
0.845	PRX9[3]			+		+	
0.890	PRX9[4]			+			
0.926	PRX10[1]			+			

In general the present study on isozyme shows genetical distinctness of each species/ population with high degree of genetical variation within the species. Maximum degree of diversity is observed in *Pteris argyraea* and the minimum degree of diversity is seen in *P. otaria* Beddome. The unrelated species *P. vittata* shows its genetical identity by having five distinct bands out of seven banding profile in this enzyme system. Lamina in *P. vittata* L. is simply pinnate in contrast to all the members of the *P. quadriaurita* complex and this morphology based classification is supported by the present isozymic profile analysis. In general simplified lamina is more advanced one when compared with the more pinnatifid fronds. Population of *P. biaurita* L. from Kodaikanal Botanical Garden is with high genetic variability with the presence of eight bands when compared with the population of the same species from Kothayar which is with only four unmatched bands. Thus both the populations show 100% genetic distinctness. The reason for such variability may be due to its cytological difference. There are several reports for the presence of both diploid and triploid apogamous cytotypes in *P. biaurita* from South India[4,5,11-13]. They are more or less similar in morphology except slight variation in the venation pattern. Triploid apogamous cytotype is more common in South India when compared with the diploid apogamous cytotype. Since the usual origin of triploid cytotype in ferns is by the hybridization between tetraploid and diploid sexual cytotypes, the triploid cytotype may have entirely different combination of genomes resulting in high degree of genetical variability as seen in the present study. *Pteris confusa* is also genetically distinct species with six bands, out of which only one band share the position with related species *P. otaria*. The minimum degree of diversity in *P. otaria* is due to its hybrid origin from two primitive diploid species *P. quadriaurita* Retz. with bipinnate frond and *P.*

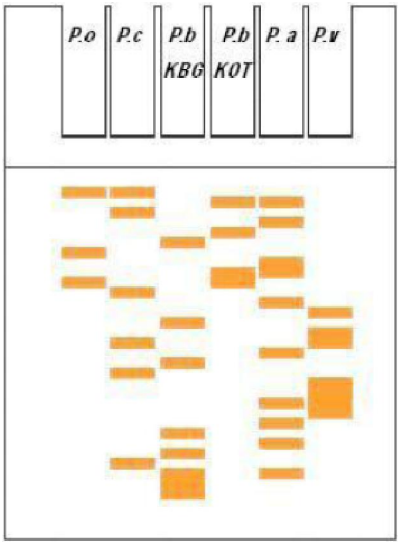

Figure 1 : Isoperoxidase profiles of *Pteris* sps. *P.o-Pteris otaria, P.c-Pteris confuse, P.b*(KBG)-*Pteris biaurita* from Kodaikanal Botanic Garden, *P.b*(Kot)-*Pteris biaurita* from Kothayar, Tirunelveli, *P.a-Pteris argyraea, P.v-Pteris vittata*

multiaurita Ag. with simply pinnate frond. *P. otaria* is with partial bipinnate frond. The sharing of one band with *P. confusa* and another band with *P. biaurita* by *P. otaria* clearly shows the common origin of all the above three species from *P. quadriaurita* Retz. Recent studies on morphology, cytology and molecular variation of *Pteris fauriei* Hieron. From Taiwan shows the presence of diploid sexual and triploid apogamous cytotypes[14] and they share a common haplotype of cpDNA apB-rbcL and trnL-trnF spacers. The ISSR markers do not show significant differentiation between these two varieties, and their genetic variation exists mainly among populations and less within the population.

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