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Studies on Tissue Specific Variation and Developmental Variation in the Isoperoxidase Pattern of the Selected Endemic Tree Species of Western Ghats

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Abstract

The present study was aimed to study the isoperoxidase pattern of the seeds and adult plant leaves of the endemic tree species viz., *Hopea utilis* Bedd. Bole, *Poeciloneuron pauciflorum* Bedd, *Gluta travancorica* Linn and *Garcinia travancorica* Bedd of Western Ghats, India. A total of 41 bands in 23 different locations with five active regions were observed in the isoperoxidase system of the selected endemic tree species. The seeds of *Hopea utilis* showed totally eight bands with different MW-Rf values viz., 0.06, 0.097, 0.131, 0.205, 0.254, 0.296, 0.356 and 0.422. Of which, the bands with MW-Rf values 0.06 and 0.356 was shared by the seeds and adult plant leaves of *Hopea utilis*. The seeds of *P. pauciflorum* collected from kannikatty showed four bands (0.051, 0.276, 0.322 and 0.402) with four active zones. The band with MW-Rf value 0.402 was shared by the seeds of *P. pauciflorum* collected from kannikatty and Inchikuzhi. The seeds of *P. pauciflorum* collected from Inchikuzhi showed only two bands with MW-Rf values 0.319 and 0.402. A total of 7 bands in six different positions with four activity regions were observed in the isoperoxidase enzyme system of *Gluta travancorica*. A total of six bands from different positions were observed in seeds of *Garcinia travancorica* and six in adult plant leaves of *G. travancorica* were observed in this enzyme system and their Rf values ranged from 0.097 to 0.484. Multiple zone of activity was observed for this enzyme system (PRX 1 to 5). The results of peroxidase activity and pattern changes in the seeds and adult plant leaves of the selected endemic tree species viz., suggest that each isoperoxidase or groups of isoperoxidase function in different capacities and over-all banding pattern of isoperoxidase may help in the identification of the endemic tree species.

1. Introduction

Morphological characters of plants play a vital role in plant systematic study and used as a tool for the classification of a taxon. Variation in morphological traits can have limited use in understanding population genetics because they are often limited in number, can be influenced by environmental conditions, and are often inherited by multiple genes. In recent days, in addition to morphological characters, anatomical, cytological, biochemical and molecular profiles are also being used to classify the taxons. The morphological and anatomical markers have several disadvantages when used as markers in botanical studies, e.g. they cannot distinguish ploidy variation, homozygotes and heterozygotes from each other. Though cytological analysis reveals the ploidy variation, chromosomal characters, it also has some disadvantage in the choice of explants and preparation of material for cytological study i.e fixation of the explants in an appropriate time (before 8 O' clock). But through biochemical analysis (Proteins and enzymes) or specifically using isoenzymes, species can be easily distinguished [1]. Isoenzymes are a powerful tool for gene variability within and between the populations of plants and animals and developmental variation, yet nowadays molecular techniques based on DNA are used. In contrast to DNA marker, isoenzyme analyses are widely used for their relative efficiency and cost - effectiveness, particularly in studies of intra and inter-specific variability [2-7]. These techniques can be more powerful than morphological markers because they allow the detection of heterogeneity among plants that appear morphologically identical [8]. The use of isozymes as genetic markers has increased dramatically over the last three decades as it has a number of important advantages over more conventional morphological markers [9]. With this background the present study was aimed to study the isoperoxidase system of the seeds and adult plant leaves of the endemic tree species viz., *Hopea utilis* Bedd. Bole, *Poeciloneuron pauciflorum* Bedd, *Gluta travancorica* Linn and *Garcinia travancorica* Bedd of Western Ghats, India. Isozyme studies would help

to elucidate the biochemical marker for the endemic tree species of the Western Ghats, South India.

2. Materials and Methods

Seeds and adult plant leaves of *Hopea utilis* Bedd. Bole, *Poeciloneuron pauciflorum* Bedd, *Gluta travancorica* Linn and *Garcinia travancorica* Bedd were used as plant materials. For peroxidase, 500 to 1000 mg of young freshly harvested leaves from adult trees and seeds of selected tree species were harvested from the wild and homogenized with 3.5 ml of ice cold homogenizing buffer (0.1M Phosphate buffer (pH 7.0)) in a pre-chilled pestle and mortar and centrifuged at 12,000 rpm for 10 min. The supernatant was subjected to electrophoresis as described by Sadasivam and Manickam [10] on PAGE. For the detection of isozymes on the gels, the staining solution, were prepared by Smila *et al.,* [11]. After the electrophoresis, the gels were incubated in the staining solution for few minutes under the dark condition till the clear bands appeared. The gels were fixed with 7% acetic acid solution for 30 min. and the gels were washed with distilled water and the bands were documented using the Vilber loubermat gel documentation system. The bands profiles were compared using the Bio-gene software (Germany).

3. Results and Discussion

A total of 41 bands in 23 different locations with five active regions were observed in the isoperoxidase system of the selected endemic tree species (Fig. 1. C and D).

Hopea utilis

With reference to *Hopea utilis* isoperoxidase system, a total of fourteen bands with five active regions were observed (Fig. 1. B). Seeds and adult plants leaves of *Hopea utilis* showed the similarity and variation in their banding profile. The seeds of *Hopea utilis* showed totally eight bands with different MW-Rf values viz., 0.06, 0.097, 0.131, 0.205, 0.254, 0.296, 0.356 and 0.422. Of which, the bands with MW-Rf values 0.06 and 0.356 was shared by the seeds and adult plant leaves of *Hopea utilis*. The adult plant leaves showed their uniqueness with varied MW-RF viz., 0.128, 0.279, 0.336 and 0.430 from seeds of *Hopea utilis* (Table - 1).

Table 1: Isoperoxidase profile of selected tree species of Western Ghats of India

MW-Rf	Position	*Hu*-Seed	*Hu* – Adult	*Pp* – Seed - In	*Pp* – Adult - In	*Pp* – Seed - K	*Glu. Tra Seed*	Glu. Tra Adult	Gar. Tra Seed	Gar. Tra Adult
0.051	PRX 1^1					+				
0.060	PRX 1^2	+	+							
0.097	PRX 1^3	+						+	+	+
0.128	PRX 2^1		+							
0.131	PRX 2^2	+								+
0.205	PRX 3^1	+							+	
0.254	PRX 3^2	+								
0.276	PRX 3^3					+		+		+
0.279	PRX 3^4		+							
0.296	PRX 3^5	+								
0.319	PRX 4^1			+	+		+			
0.322	PRX 4^2					+			+	+
0.336	PRX 4^3		+							
0.356	PRX 4^4	+	+							+
0.385	PRX 4^5						+	+		
0.387	PRX 4^6								+	
0.399	PRX 4^7				+					
0.402	PRX 5^1			+		+				
0.422	PRX 5^2	+							+	+
0.430	PRX 5^3		+					+		
0.484	PRX 5^4								+	
0.487	PRX 5^5						+			

In – Inchikuzhi; K – kannikatty;

Poeciloneuron pauciflorum

The *Poeciloneuron pauciflorum* isoperoxidase system showed a total of eight bands with four active zones (Fig. 1. B). Zone two was not shown any band in this system. The *P. pauciflorum* isoperoxidase system includes the seeds and adult plant leaves of *P. pauciflorum* collected from Inchikuzhi and Seeds of *P. pauciflorum* collected from kannikatty. The seeds collected from Inchikuzhi and kannikatty showed the different

banding profile. The band with MW-Rf value 0.402 was shared by the seeds of *P. pauciflorum* collected from kannikatty and Inchikuzhi. The seeds of *P. pauciflorum* collected from kannikatty showed four bands (0.051, 0.276, 0.322 and 0.402) with four active zones. The seeds of *P. pauciflorum* collected from Inchikuzhi showed only two bands with MW-Rf values 0.319 and 0.402. The adult plant leaves of *P. pauciflorum* collected from Inchikuzhi showed only two bands with MW-Rf values 0.319 and 0.399. The band 0.319 was commonly present in seeds and adult plant leaves of *P. pauciflorum* collected from Inchikuzhi (Table - 1).

Gluta travancorica

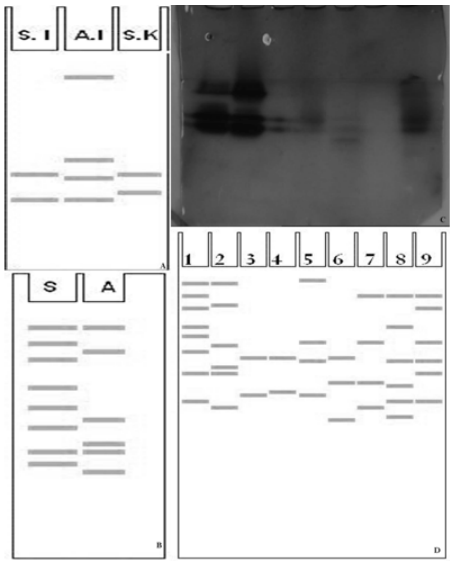
A total of 7 bands in six different positions with four activity regions were observed in the isoperoxidase enzyme system of *Gluta travancorica*. Zone two was not shown any band in this system. Region one contained only one band PRX 1^1(0.097) present in adult plant leaves of *G. travancorica* with restriction. Region three showed only one band in this enzyme system with the MW-Rf value 0.276 (PRX3^3). Region four expressed with two bands in the isoperoxidase system of *G. travancorica*. PRX 4^1(0.319) was showed their unique presence in the seeds of *G. travancorica*, PRX 4^5(0.385) was shared by the seeds and adult plant leaves of *G. travancorica*. Region five expressed two bands with two different Rf values 0.430 and 0.487. PRX 5^3 (0.430) was unique to adult plant leaves of *G. travancorica*; PRX 5^2 (0.487) for seeds of *G. travancorica* (Table - 1).

Garcinia travancorica

A total of six bands from different positions were observed in seeds of *Garcinia travancorica* and six in adult plant leaves of *G. travancorica* were observed in this enzyme system and their Rf values ranged from 0.097 to 0.484. Multiple zone of activity was observed for this enzyme system (PRX 1 to 5). Zone one showed only one band (PRX 1^1) with the 0.097 banding position was observed. The first band (PRX 1^1) was common to seeds and adult plant leaves of *G. travancorica*. Similar to zone one, Zone two also contained only one band (PRX2^2 – 0.131). The band (PRX2^2 – 0.131) showed their presence only in adult plant leaves of in *G. travancorica*. In Zone 3, a total of two bands were observed in different positions (PRX 3^1and3^3). The first band was restricted to seeds of *G. travancorica*. This unique banding profile can be used as identification marker for this species. The second band (PRX 3^3) was observed in adult plant leaves of *G. travancorica*. Zone 4 contained four bands in three different positions; the first band (PRX 4^2) showed their presence in seeds and adult plant leaves of *G. travancorica*. The second band (PRX 4^4) was expressed only in adult plant leaves of *G. travancorica*. PRX 4^6 (0.387) was present only in seeds of *G. travancorica*. Zone 5 showed three bands in two different positions. The band (PRX 5^2) was common to seeds and adult plant leaves of *G. travancorica*. The band (PRX 5^4) showed their presence only in seeds of *G. travancorica* (Table - 1). .

The changing pattern in seeds and adult plant leaves may be interpreted as evidence for differential timing of gene expression correlated with the physiological changes during the development [11]. The explants seeds and leaves of the selected tree species displayed a characteristic banding pattern in the isoperoxidase system that can be compared with other tree species for the identification (Fig. 1 C and D; Table – 1). Each zone is occupied by a particular isozyme in the form of a band and is representative of the expression of a particular gene locus coding for that isozyme. In certain stages, in a particular zone more than one distinct band is resolved. The general pattern of appearance and disappearance of bands can be explained similarly on the basis of gradual shifts of isozyme patterns in samples taken in the course of development due to differential activation of genes involved in synthesis of these enzymes at different stages of development [9]. Smila et al., [11] observed that the polymorphism and genetic expression at different developmental stages in *Lens culinaris* and *Pennisetum glaccum* using the isoenzymes (esterase and acid phosphatase). The seeds and adult plant leaves of the selected endemic tree species showed the variation in the banding profile. Unique banding patterns of isoperoxidases were observed in the seeds and adult plant leaves of the selected trees of the Western Ghats, which represented the "Finger Print" of that particular endemic tree species. Genetic variation is the fundamental component of adaptation and thus, of stability of forest ecosystems. This is particularly important when the long-term stability of forest ecosystems is increasingly threatened by environmental stress and mismanagement. During the past 20 years, enzyme electrophoresis has been used to describe the population genetic structure of over 700 plant taxa [12]. Variation is the basic resource to be explored for genetic improvement in any species and hence play a key role in plant improvement programmes [13-15]. Many researchers have studied the genetic variability in inter and intra-populations on natural ecosystems for the purposes of gene pool conservation [16-17]. Morphological characteristic might themselves be insufficient to distinguish between pairs of closely related species, geographical races, or ecotypes, because not all-genetic differentiation results in morphological-differentiation.

Fig. 1. Isoperoxidase pattern of the Selected Endemic Trees Species of the Western Ghats, India



A. *Poeciloneuron pauciflorum* (SI - Seed from Inchikuzhi; AI – Adult plants leaves from Inchikuzhi; SK- Seed from Kannikatty), B. *Hopea utilis* (S – Seeds; A - adult plants leaves), C. Isoperoxidase pattern of the *Hopea utilis, Poeciloneuron pauciflorum, Gluta travancorica* and *Garcinia travancorica* and *D.* Zymogram of the *Hopea utilis, Poeciloneuron pauciflorum, Gluta travancorica* and *Garcinia travancorica* (1- *Hopea utilis* Seed; 2 - *Hopea utilis* Adult plant leaves; 3 - *Poeciloneuron pauciflorum* seed from Inchikuzhi; 4 - *Poeciloneuron pauciflorum* adult plant leaves from Inchikuzhi; 5 - *Poeciloneuron pauciflorum* seed from Kannikatty; 6 - *Gluta travancorica* seed; *7- Gluta travancorica* Adult plant leaves; 8 - *Garcinia travancorica* seeds and 9 - *Garcinia travancorica* Adult plant leaves).

The results of peroxidase activity and pattern changes in the seeds and adult plant leaves of the selected endemic tree species viz., suggest that each isoperoxidase or groups of isoperoxidase function in different capacities and over-all banding pattern of isoperoxidase may help in the identification of the endemic tree species. Further molecular studies on DNA using ISSR, SSR and VNTRs may provide good outcome and strengthen the identification of the endemic tree species.

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