STUDY OF ALLOZYMES IN LEUCAS BIFLORA (VAHL.) R. BR. OF LAMIACEAE.

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Abstract

In the present investigation, Plant allozymes of family Lamiaceae (*Leucas biflora*) have been studied to establish the Phylogenetic relationship. In the present study five allozymes were tested Viz. Superoxide dismutase, Succinate Dehydrogenase, Polyphenol Oxidases, Catalase and Rubisco and data was interpreted by using STATISTICA package. Four enzymes showed reliable polymorphism except Catalase. In Catalase monomorphic allele was elucidated. The bands were stained and allele frequency was calculated. The difference in allele frequency can be helpful to the taxonomist in establishing genetic diversity amongst the taxa of Lamiaceae.

Keywords- Allozymes, Zymogram, Allele frequency, Polymorphism, Phylogeny.

Introduction:

The most widespread in its distribution amongst Plant kingdom is Angiosperms. "Tubiflorae" is the largest order of Engler and Diels (1936), revised system of classification with primarily herbaceous plants, gamopetalous corolla, epipetalous stamens. Taxonomists treated the order Tubiflorae differently. The variation in allele frequency would support as an additional taximetrics in deciding the position of the families like Acanthaceae, Lamiaceae, Scrophulariaceae and Lentibulariaceae.

Allozymes share a common substrate but differ in their electrophoretic mobility that helps in making the comparisons between the populations. In the present investigation, efforts have made to support the new taximetrics on the basis of Isozyme pattern and shared loci.

Allozyme pattern has been studied for calculating the percentage of gene loci and polymorphism per population. For out crossing plants, the numbers and frequencies of alleles detected in any one population are often very similar to another population of the taxa belonging to same family studied. Thus, the findings in the present investigation proved to be helpful to justify the position of the family in the order Tubiflorae on molecular basis.

Review of Literature

Meister (1950), studied the taxa to understand the molecular heterogenesity. Markert (1975a, b, c) published the information about isozymes in 3 volumes namely Isozymes –I, Isozymes –II, &Isozymes –III. Sonnante et al. (1997) obtained better insight into genetic relationship within and between the taxonomic entities of *Vigna luteola* and *V. marina*. Bhat et al. (1998) studied the Isozyme diversity in Indian primitive maize landraces and observed polymorphism for Peroxidase, esterase and acid phosphatase. Apavatjrut et al. (1999) carried out the study on *Curcuma* species. Volis et al. (2003), interpreted that allozyme variation in wild barley is adaptive and directly related to local environment. Soltis & Soltis (2005) gave the detailed study of isozymes in different chapters of plant biology. Isozyme used as a molecular marker to assessed the genetic diversity and structure of wild Tunisian *Thymus capitatus* of Lamiaceae (Ali et. al.2011). Gömöry *et. al.* focussed on potential discordances in spatial patterns of allozyme and quantitative phenotypic variation.

Materials and Methods

The plant material was collected from localities in and around Nagpur District. The seeds of *Leucas biflora* were randomly collected. They were sun-dried and investigation was carried out with fresh material as well as water soaked viable seeds. Band variation was studied as per the method given by Sadashivam and

Manickam, 1996; Vellejos, 1983; Wendel & Weeden, 1989.The gel was photographed and interpreted by using the "STATISTICA" package.

Observations

The taxa under investigation showed reliable polymorphism except Catalase due to variation in the banding pattern. Allozyme data was analyzed through cluster analysis by simple matching coefficient method. 27 significant alleles were resolved, of which, number of alleles (bands) found in Rubisco i.e. eight, next to it is seven in Amylase followed by two in Alcohol Dehydrogenase. Catalase is the only monomorphic showing single allele. Zymogram of Rubisco is explained in the given table and scored as follows-

The isozyme pattern of Rubisco is explained below:

T *Lb*

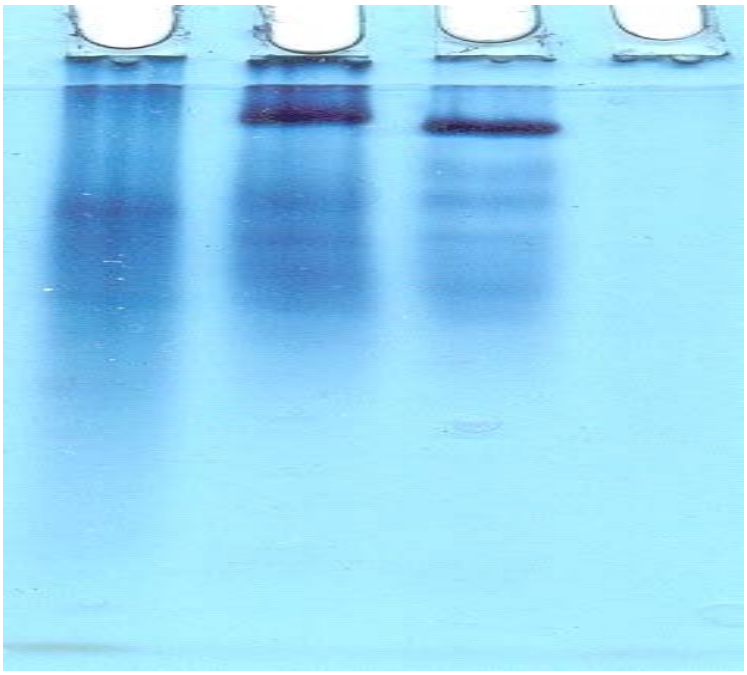

Fig: 1- Zymogram of of Rubisco

Table 1:

Name of Enzyme	**Alleles**	A	***Leucas***	B	C	**% of shared loci(P)**
Rubisco	1	1.000	1.000	1.000	0.000	75.000
Rubisco	2	0.000	1.000	1.000	0.000	50.000
Rubisco	3	0.000	0.000	1.000	0.000	50.000
Rubisco	4	1.000	1.000	1.000	1.000	100.000
Rubisco	5	1.000	1.000	1.000	0.000	75.000
Rubisco	6	1.000	1.000	1.000	0.000	75.000
Rubisco	7	0.000	1.000	1.000	0.000	50.000
Rubisco	8	0.000	0.000	1.000	0.000	25.000
Allele Frequency		**0.500**	**0.750**	**1.000**	**0.125**	

The zymogram represents various isozymes of Rubisco. Figure:1 shows 8 bands of four samples species(A,B,C)along with Leucas biflora studied for isozymes. The intensity of band indicates the amount of isozyme present in the sample loaded for electrophoresis. Rubisco 1,4,5,6 present in three samples. Rubisco 2, 7 reported as a single band in two taxa, while 8 in only sample C. 3 bands are shared by Maximum Species. The presence and the absence of band is stated as 1 or 0 respectively and the data is tabulated in Table- I & II. The percentage of population per sample sharing each band /allele/ locus has been calculated below.

The example studied is Rubisco. In Fig. 2 the first locus is present in 4th samples and hence the distribution % among the population is 4/6 x 100 = 66.667. Second in only one sample and hence distribution % is 1/6 x 100 =16.667. Similarly the allele frequency is calculated for all the samples on the basis of enzyme locus present out of the total detected isozyme loci. Thus, the allele frequency of Gantelbua, Leucas and Utricularia is same i.e. 500, as far as the band pattern and sharing of loci is concern. The taxon showing more than one band is said to be polymorphic where as only one band is monomorphic by nature. The isozyme scoring and relative frequency in the taxa investigated is given in Table III.

T *Lb*

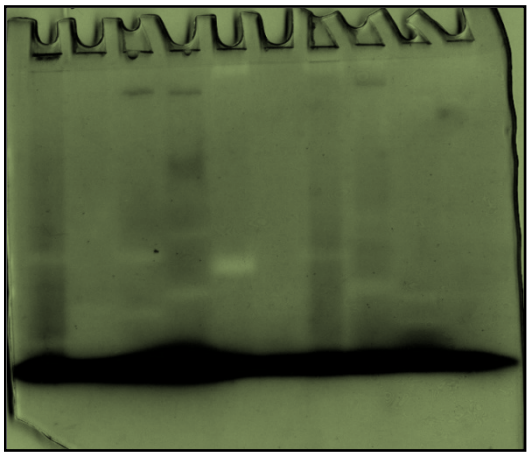

Fig2: Superoxide dismutase

T *Lb*

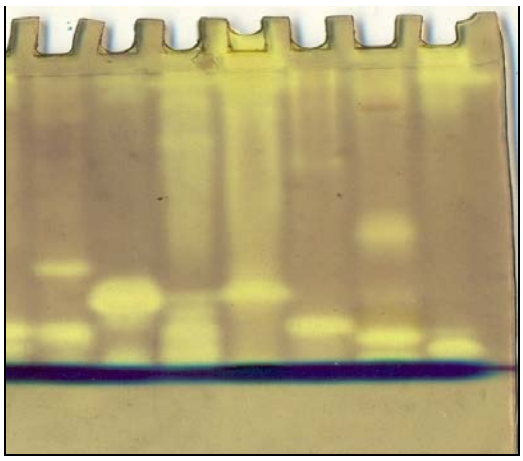

Fig3: Succinate Dehydrogenase

T *Lb*

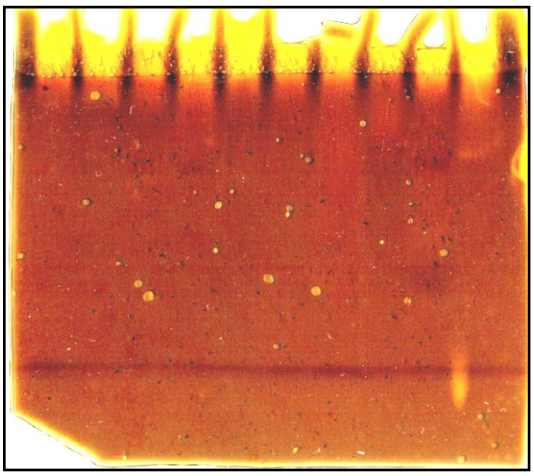

Fig3: Catalase

T *Lb*

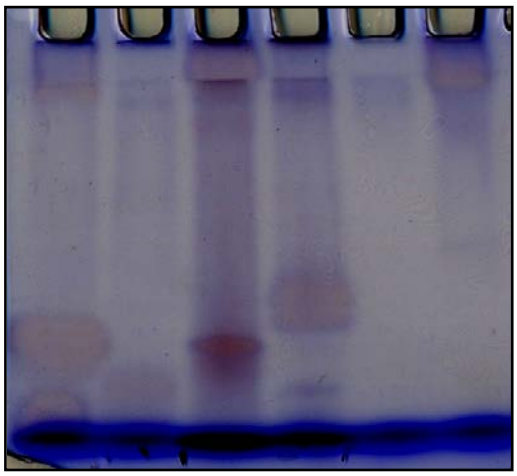

Fig4: Polyphenol Oxidases

The Rubisco shows 8 bands recorded in three taxa and roughly recorded in single taxon. The intensity of band indicates the amount of isozyme present. Rubisco 1 is shared by maximum Species present in all the four samples. Rubisco 1, 4, 5 & 6 reported in maximum taxa while 3 and 7 shared by only 2 taxa whereas 8 shared by only one percent population. In enzyme catalase, the first locus is present in all the four samples and hence the

distribution % among the population is 4/4 x 100 = 100%. The allele frequency is calculated for all the samples on the basis of enzyme locus present out of the total detected allozyme loci. Thus, the allele frequency calculated of *Leucas biflora* is helpful when it is compaired with the related data collected from the remaining taxa of same family. The band pattern and sharing of loci is concern. The taxon showing more than one band is said to be polymorphic where as only one band is monomorphic by nature. The isozyme scoring and relative frequency of the four other enzymes are given below:

Table: II

Name of Enzyme	A	***Leucas***	B	C	**% of shared loci(P)**
Catalase	1.000	1.000	1.000	1.000	100.000
Allele Frequency	**1.000**	**1.000**	**1.000**	**1.000**	
Polyphenol Oxidases	1.000	1.000	0.000	0.000	50.000
	1.000	1.000	0.000	1.000	75.000
	1.000	0.000	0.000	0.000	25.000
	0.000	0.000	0.000	1.000	25.000
	0.000	1.000	0.000	0.000	25.000
	0.000	1.000	0.000	0.000	25.000
	1.000	0.000	0.000	0.000	25.000
	1.000	1.000	1.000	1.000	100.000
Allele Frequency	**0.500**	**0.600**	**0.100**	**0.300**	
Superoxide dismutase	1.000	0.000	0.000	1.000	50.000
	0.000	0.000	1.000	1.000	50.000
	1.000	0.000	0.000	0.000	25.000
	0.000	0.000	0.000	0.000	00.000
	0.000	1.000	1.000	0.000	50.000
	0.000	0.000	1.000	0.000	25.000
Allele Frequency	**0.333**	**0.167**	**0.500**	**0.333**	
Succinic Dehydrogenase	1.000	0.000	0.000	1.000	50.000
	1.000	0.000	0.000	1.000	66.667
	1.000	0.000	0.000	0.000	25.000
	0.000	0.000	0.000	0.000	0.000

	0.000	1.000	0.000	0.000	25.000
	0.000	0.000	1.000	0.000	25.000
	1.000	0.000	1.000	0.000	50.000
	0.000	0.000	0.000	0.000	0.000
	0.000	1.000	0.000	0.000	25.000
	0.000	0.000	1.000	1.000	50.000
	0.000	0.000	0.000	1.000	25.000
	1.000	1.000	1.000	0.000	75.000
Allele Frequency	**0.417**	**0.250**	**0.333**	**0.333**	

Table II shows the total allele frequency in all the four taxa studied, depicting the affinity between them. It is calculated on the basis of total allozymes detected.

Discussion and Conclusions:

The investigation carried out could be helpful to find the co-relation among different families which shows affinities and to discuss the link between them. Here one taxon i.e. only Leucas biflora has been studied for five different plant enzymes so as to know about how far this data is reliable to establish the link between close families like Acanthaceae, Lamiaceae, Scrophulariaceae and Lentibulariaceae.

The use of new cladistics in taxonomy for ascertaining taxonomic similarities is recent at infra specific, generic and family level. In this aspect, the present investigation could be helpful for the taxonomists in ascertaining the new data. Earlier, in nineteenth century many taxonomists make use of phytochemical investigations, cellular details and took support of the embryological findings. The isozymes have been used for the first time as a tool in the identification of some *Curcuma* species (Apavatjrut et al., 1999). Molecular markers proved to be effective in presenting the reliable data in deciding the positions of certain families. The isozyme data in the present investigation has been proved to be helpful in making the taxonomic clusters. For eg. One is of Acanthaceae and Scrophulariaceae and second of Lamiaceae and Lentibulariaceae .

Lange and SchifinoWittmann (2000), Batista and Sosa (2002), Fu and Dane (2003), Mateu-Andres (2004), Gonzalez Astorga et al. (2004) and Jaaska (2005). Das and Mukherjee (1997), and Kofi et al (2009) combinely analysed the isozyme data to confirm the taxonomic alignment. The isozyme data in the present investigation has been proved to be helpful in making the taxonomic clusters. For eg. One is of Acanthaceae and Scrophulariaceae and second of Lamiaceae and Lentibulariaceae .

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