



## Isozyme Analysis of Some Families of Tubiflorae

S. Dakhore<sup>1</sup> and N. Dongarwar<sup>2</sup>

<sup>1</sup>Department of Botany, Kamla Nehru Mahavidyalaya, Nagpur

<sup>2</sup>Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur -440033.  
dmnitin26@gmail.com

### Abstract:

Plant isozymes are used as a tool to decide the affinities among the family of "Tubiflorae" *Volvulopsis nummularius* of Convolvulaceae, *Leucas biflora* of Lamiaceae, *Lindernia crustacea* of Scrophulariaceae and *Utricularia aurea* of Lentibulariaceae have been studied and zymogram was obtained. In the present study four isozymes were tested (Succinic Dehydrogenase, NAD-Diaphorase, Esterase, Acid Phosphatase) and data was interpreted by using STATISTICA package. All the four enzymes studied shows reliable polymorphism. The bands were stained specifically and allele frequency was calculated. The difference in allele frequency is responsible for genetic diversity between the families of Tubiflorae.

**Keywords:** Tubiflorae, Isozymes, Zymogram, Allele frequency, Polymorphism.

### Introduction:

Angiosperms, a highly evolved group of Plant kingdom are widespread in its distribution and also exhibit the dominant vegetation in the world. "Tubiflorae" comprises primarily herbaceous plants with gamopetalous corolla, characterized by the floral parts usually in 4 isomerous whorls, stamens epipetalous, pistil hypogynous and ovules are unitegmic. Taxonomists treated the order Tubiflorae and its constituent families differently e. g. Series Bicarpellatae under Gamopetalae include majority of the families of Tubiflorae. The revised system of Engler and Diels (1936) recognized the order "Tubiflorae" under the Class-Dicotyledonae and subclass sympetalae of Metachlamydeae. The families studied here are Convolvulaceae, Lamiaceae, Scrophulariaceae and Lentibulariaceae.

Isozymes or Allozymes are the enzymes that share a common substrate but differ in their electrophoretic mobility. It helps in making the comparisons between individuals and population on the basis of several gene loci. In the present investigation, by using this molecular tool, efforts are made to establish the link between some families of order 'Tubiflorae'. Further, to demonstrate the relationship of a single genus studied from each family with other genera of the families. On the basis of allele frequency, 4 sets of population were studied and the shared gene pool was calculated. On the basis of Isozyme pattern and shared loci, it was concluded that the taxa under investigation are poorly co-related with each other. The electrophoresis technique gives morphology of Isozyme pattern i.e. chromatic or achromatic bands, monomorphic or polymorphic conditions ascertain the number of isozymes.

While studying the isozyme pattern, measures of genetic variation including percentage of gene loci polymorphism per population has been considered. 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 studied. Thus, the findings in the present investigation proved to be helpful to justify the position of the families of the order Tubiflorae on molecular basis.





## Review of Literature:

The four genera of order “Tubiflorae” have been investigated to understand the molecular heterogeneity (Meister, 1950). The study of isozyme and their recognition is relatively recent, being first announced in 1959 (Markert 1968). Markert (1975a, b, c) published the information about isozymes in 3 volumes in Isozymes –I, Isozymes –II, and Isozymes –III. Farinelli et al. (1983) have isolated, purified and partially characterized formate dehydrogenase from Soybean seed as its study offer several advantages in the field of biotechnology. Elisens and Nelson (1993) have studied the morphological and isozyme divergence in *Gambelia* of Scrophulariaceae. Sonnante et al. (1997) obtained better insight into genetic relationship within and between these taxonomic entities of *Vignaluteola* and *V. marina*. Isozyme diversity in Indian primitive maize landraces were studied by Bhat et al. (1998) and observed polymorphism for Peroxidase, esterase and acid phosphatase isozymes. Apavatjirut et al. (1999) used isozyme for the first time as tools to aid identifications of some *Curcuma* species which are still taxonomically confused. Lange and Schifino-Wittmann (2000) evaluated the isozyme patterns for Phosphoglucosomerase, Malate Dehydrogenase, Esterase and Superoxide Desmutase of eight *Trifolium* species. Volis et al. (2003) interpreted that allozyme variation in wild barley is adaptive and directly related to local environment. Mateu- Andres (2004) studied the allozyme variability in the threatened species of *Antirrhinum subbaeticum* and *A. pertegasu* of Scrophulariaceae. Soltis and Soltis (2005) gave the detailed study of isozymes in different chapters of plant biology. VZaouali and Boussaid (2008) have made a comparative analysis of population structure by studying isozyme markers and volatiles in Tunisian *Rosemarinus officinalis* of Lamiaceae. Ali et al. (2011) used the isozyme markers and assessed the genetic diversity and structure of wild Tunisian *Thymus capitatus* of Lamiaceae. Chang et al. (2012) observed the effect of cadmium on peroxidase isozyme activity in roots of two *Oryza sativa* cultivars.

## Material and Methods:

The plant material was collected from localities in and around Nagpur District. For isozyme study the taxa namely *Leucasbiflora*, *Lindernia crustacea*, *Volvulopsis nummularia* and *Utricularia aurea* were selected. The seeds from species were randomly collected from each population. They were sun-dried and stored as germplasm at room temperature. Thus, each collection of seed samples represents the total gene pool of individual population. Investigation was carried out on fresh as well as water soaked viable seeds. Isozymes studies were conducted as per the method given by Sadasivam and Manickam, 1996; Vellejos, 1983. The gel was photographed immediately and interpreted by using the “STATISTICA” package.

## Observations:

The taxa belonging to Convolvulaceae, Lamiaceae, Scrophulariaceae and Lentibulariaceae of Tubiflorae have been studied at molecular level showed reliable polymorphism. Isozyme data was analyzed using cluster analysis of simple matching coefficient and UPGMA to produce a dendrogram depicting the degree of relationship among the species (Apavatjirut, et al, 1999). 36 significant alleles were





resolved, of which, the highest number of alleles (bands) found is twelve in Succinic Dehydrogenase, next to it is nine in Esterase followed by eight in NAD-Diaphorase and seven in Acid Phosphatase. The isozyme pattern of NAD Diaphorase is explained (Table - III) and the zymograms are scored (Figure 1-4)

The zymogram represents various isozymes of four enzymes. For Ex. NAD Diaphorase shows 8 bands of samples species studied for isozymes and recorded in two taxa. The intensity of band indicates the amount of isozyme present. NAD Diaphorase 1 present in two samples i. e. *Lindernia* of Scrophulariaceae and *Utricularia* of Lentibulariaceae. NAD Diaphorase 2, 4 reported as a single band in *Lindernia* and *Utricularia*, while 2 and 7 in *Leucas*. 3 bands are shared by Maximum Species. The percentage of population per sample sharing each band /allele/ locus has been calculated below. The first locus is present in 2 samples and hence the distribution % among the population is  $2/4 \times 100 = 50\%$ . Second in only one sample and hence distribution % is  $1/4 \times 100 = 25\%$ . 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 *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 for Succinate Dehydrogenase is given in Table 1.

On the basis of isozyme analysis (Table I), the dendrogram was prepared which shows correlation between the taxa studied (Fig. 5)

The molecular findings also show three distinct clusters where cluster one is Scrophulariaceae showing the matching coefficient of nearly 45% and close to this cluster is the family Lentibulariaceae with 47% of matching coefficient. The slight deviation of the Lamiaceae with these two families by 57% form cluster two whereas family Convolvulaceae is completely deviated from remaining three families with 67% forming cluster three. Thus the cluster analysis displayed on molecular data purely shows close similarity between the families, Lentibulariaceae, Scrophulariaceae.

## Discussion:

In order to resolve the issue of the relationship of different families included in the Tubiflorae, molecular analysis have been carried out to discuss the relationship between the taxa. The families studied in the present investigation are Convolvulaceae, Lamiaceae, Scrophulariaceae and Lentibulariaceae. One taxa have been studied belonging to each family and accordingly relationship has been discussed among the families.

Several families have been included or removed time to time by using different tools. Taxonomists, from the long time, have tried to use the new cladistics in taxonomy for ascertaining taxonomic similarities at infra specific, generic and family level. In 1960's many taxonomists make use of phytochemical investigations as a tool to aid identification for supporting 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) that are still taxonomically confused.

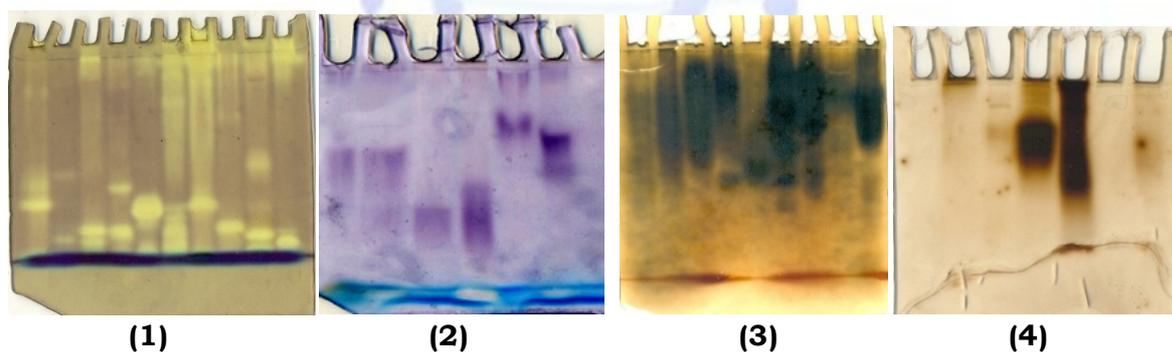


These markers were used to confirm the taxonomic identification and to distinguish the taxa analysed.

Isozyme profile under molecular study constructed the dendrogram by UPGMA shows the presence of three clusters. One is of Scrophulariaceae and Lentibulariaceae, second of Lamiaceae and third of Convolvulaceae. On the basis of molecular data family Scrophulariaceae and Lentibulariaceae are close to each other whereas Lamiaceae form separate line of cluster and Convolvulaceae is much more deviated. The same UPGMA analysis have been worked out to understand the taxonomic affinities by Apavatjirut et al. (1999), Lange and Schifino Wittmann (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) combinelyanalysed the morphological and isozyme data to estimate the taxonomic altignment. The present isozyme investigation deviates the familyonvolvulaceae and Lamiaceae from Scrophulariaceae, Lentibulariaceae confirms the separate lineage.

**Table. 1-**

Name of Enzyme	Alleles	<i>Evolvulus</i>	<i>Leucas</i>	<i>Lindernia</i>	<i>Utricularia</i>	% of shared loci (P) of shared
<i>Succinic Dehydrogenase</i>	1	1.000	0.000	0.000	1.000	50.000
	2	1.000	0.000	0.000	1.000	50.000
	3	1.000	0.000	0.000	0.000	25.000
	4	0.000	0.000	0.000	0.000	0.000
	5	0.000	1.000	0.000	0.000	25.000
	6	0.000	0.000	1.000	0.000	25.000
	7	1.000	0.000	1.000	0.000	50.000
	8	0.000	0.000	0.000	0.000	0.000
	9	0.000	1.000	0.000	0.000	25.000
	10	0.000	0.000	1.000	1.000	50.000
	11	1.000	0.000	0.000	1.000	50.000
	12	1.000	0.000	1.000	0.000	50.000
<b>Allele Frequency</b>		<b>0.500</b>	<b>0.166</b>	<b>0.333</b>	<b>0.333</b>	

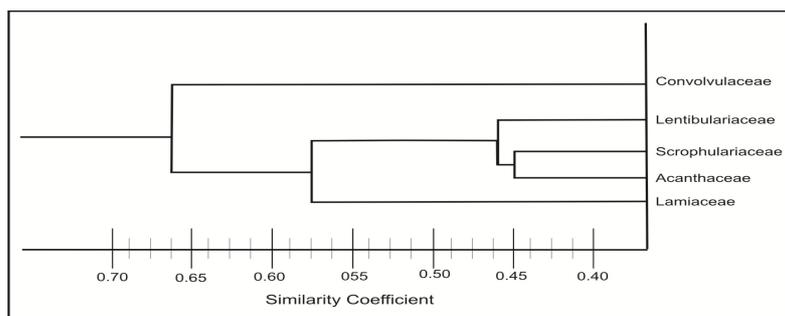


**Figure. 1-** Succinic Dehydrogenase;

**Figure. 2 -**NAD Diaphorase;

**Figure. 3-** Esterase

**Figure. 4-** Acid Phosphatase



**Figure. 5-** Dendrogram showing relationship amongst four families of Tubiflorae by using similarity coefficient of Total Allele Frequency shown by different Isozymes.

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