



Allozyme Detection in Few families of Tubiflorae.

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Abstract:

Plant allozymes are used as a tool to decide the affinities among the families of "Tubiflorae". *Volvolvopsis 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 allozymes were tested viz. Catalase, Alcohol Dehydrogenase, Amylase & Rubisco and data was interpreted by using STATISTICA package. Out of four enzymes studied three shows reliable polymorphism except Catalase where monomorphic allele was elucidated. The bands were stained specifically and allele frequency was calculated. The difference in allele frequency helped in understanding the genetic diversity between the families of Tubiflorae.

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

Introduction

A highly evolved group amongst Plant kingdom is the Angiosperms. They are widespread in distribution and also exhibit the dominant vegetation in the world. "Tubiflorae" is the largest order of dicots. Engler and Diels (1936) revised system of classification with primarily herbaceous plants, gamopetalous corolla, stamens epipetalous, pistil hypogynous and unitegmic ovules. Taxonomists treated the order Tubiflorae and its constituent families differently. The allozyme study has been carried out to support the other taxometrics in deciding the position of the families Convolvulaceae, Lamiaceae, Scrophulariaceae and Lentibulariaceae.

Isozymes or Allozymes are the enzymes that share a common substrate but differ in their electrophoretic mobility. The difference in the electrophoretic mobility helps in making the comparisons between the populations. In the present investigation, by using this molecular tool, efforts have 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 family. 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 allozyme pattern whether chromatic or achromatic, monomorphic or polymorphic ascertaining the number of allozymes.

While studying the allozyme pattern, the percentage of gene loci and 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 as under.

Review of Literature

The taxa have been studied to understand the molecular heterogeneity. Allozyme or isozyme study is relatively recent, being first announced in 1959 (Markert 1968). Markert (1975a, b, c) published the information about isozymes in 3 volumes namely Isozymes -I, Isozymes -II, & Isozymes -III. 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 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. The identifications of some *Curcuma* species which are still taxonomically confused was carried out by using the recent tool by Apavatjirut et al. (1999). Volis et al. (2003) interpreted that allozyme variation in wild barley is adaptive and directly related to local environment. The allozyme variability in the threatened species of *Antirrhinum subbaeticum* and *A. pertegasu* of Scrophulariaceae was studied by Mateu- Andres (2004). Soltis & Soltis (2005) gave the detailed study of isozymes in different chapters of plant biology. Ali et al. (2011) used the isozyme markers and assessed the genetic diversity and structure of wild Tunisian *Thymus capitatus* of Lamiaceae. D. Gömöry et al. focused 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 from

species *Leucas biflora* (Vahl) R. Br.,
Lindernia crustacea (Linn.) F. Muell,
Evolvulus nummularia (Linn.)
 Roberty and *Utricularia aurea* Lour were
 randomly collected. They were sun-dried and
 thus representing the total gene pool of
 individual population. Investigation was
 carried out with fresh material as well as water
 soaked viable seeds. Allozymes studies were
 conducted as per the method given by
 Sadasivam and Manickam, 1996; Vellejos,
 1983; Wendel & Weeden, 1989. The gel was
 photographed immediately and interpreted by
 using the "STATISTICA*" package.

Observations

The taxa under investigation showed reliable
 polymorphism except Catalase. Allozyme data
 was analyzed using cluster analysis of simple
 matching coefficient. 18 significant alleles were
 resolved, of which, the highest number of
 alleles (bands) found in Rubisco i.e. eight, next
 to it is seven in Amylase followed by two in
 Alcohol Dehydrogenase and one in Catalase
 showing monomorphism. The allozyme pattern
 of Rubisco is explained in the given table and
 the zymograms are scored as follows-

VnLbLcUaVnLbLcUa

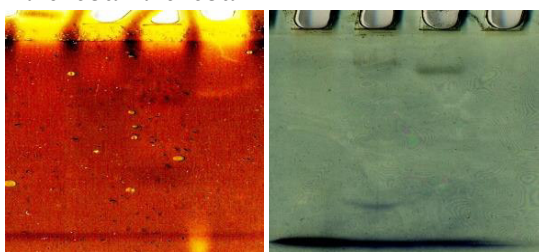


Fig1: - Catalase Fig2: - Alcohol

Dehydrogenase

VnLbLcUaVnLbLcUa

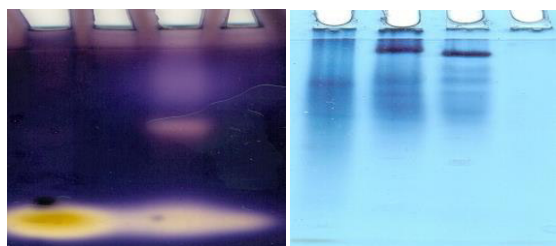


Fig3: - Amylase Fig4: - Rubisco

\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 2, 4, 5 & 6 reported in three
 taxa i.e. *Evolvulus*, *Leucas* & *Lindernia*, while 3
 and 7 shared by *Lindernia* only whereas 8
 shared by fifty percent population. The
 percentage of population per sample sharing
 each band /allele/ locus has been calculated

below. The first locus is present in all the four
 samples and hence the distribution % among
 the population is $4/4 \times 100 = 100\%$.
 Second, fourth, fifth & sixth in three samples
 and hence distribution % is $3/4 \times 100 = 75\%$.
 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 of *Leucas* and *Utricularia*
 is near about same as far as the band pattern
 and sharing of loci is concern. The taxon
 showing more than one band is said to be
 polymorphic whereas only one band is
 monomorphic by nature. The isozyme scoring
 and relative frequency in the taxa investigated
 for Rubisco is given in Table I.

Table:I

Name of Enzy	All ele s	Evol vulus	Le uc as	Lind erni a	Utric ulari a	% of sha
Rubi sco	1	1.00 0	1.0 00	1.00 0	1.000	100 .00
	2	1.00 0	1.0 00	1.00 0	0.000	75. 000
	3	0.00 0	0.0 00	1.00 0	0.000	25. 000
	4	1.00 0	1.0 00	1.00 0	0.000	75. 000
	5	1.00 0	1.0 00	1.00 0	0.000	75. 000
	6	1.00 0	1.0 00	1.00 0	0.000	75. 000
	7	0.00 0	0.0 00	1.00 0	0.000	25. 000
	8	1.00 0	0.0 00	1.00 0	0.000	50. 000
Allel e Freq uenc y	Ru bis co	0.75 0	0.6 25	1.00 0	0.12 5	

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.

Table:II

	Name of enzyme	Evolvulus	Leucas	Lindernia	Utricularia
Allele Frequency	Rubisco	0.750	0.625	1.000	0.125
	Catalase	1.000	1.000	1.000	1.000
	Alcohol Dehydrogenase	1.000	0.500	1.000	0.500
	Amylase	0.429	0.286	0.571	0.429
Total allele Frequency	04	3.179	2.411	3.571	2.054

Discussion and Conclusions

The present work has been carried out to find the relationship among different families included in the Tubiflorae and to discuss the link between them. One taxon from each family has been studied so as to establish the link accordingly among them. The families are Convolvulaceae, Lamiaceae, Scrophulariaceae and Lentibulariaceae. 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. The present investigation could be helpful for the taxonomists in ascertaining the new data. In the early nineteenth century many taxonomists make use of phytochemical investigations and support the embryological findings. The isozymes have been used for the first time as a tool in the identification of some *Curcuma* species (Apavatjirut et al., 1999). These markers were used to confirm the taxonomic identification and to distinguish the taxa analysed. Further the cluster analysis shows presence of two clusters. One is of Scrophulariaceae and Convolvulaceae and second of Lamiaceae and Lentibulariaceae. The same 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) combinely analysed the morphological and isozyme data to estimate the taxonomic alignment. The present isozyme investigation deviate the family Convolvulaceae & Scrophulariaceae, from Lamiaceae and Lentibulariaceae confirms the separate lineage.

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