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Phylogenetic analysis by using isozymes

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Abstract
A few number *Tephrosia* species have been recorded to have morphological complexity which could cause the problem to the work of taxonomist to make decisions. In order to support taxonomist a new experimental method using SDS-PAGE will be used to explore isozyme data the main purpose of this research is whether isozyme data can be used to clarify morphological differences. The present study characterizes three species of *Tephrosia* through its biochemical analysis including protein and polyacrylamide gel electrophoresis the increasing concentration of protein was observed in the order like *Tephrosia pentaphylla, Tephrosia villosa* and *Tephrosia purpurea*. Two isozyme systems have been recorded from the *Tephrosia* species alkaline phosphatase and esterases along with that molecular Weight determination was carried out as these species are economically important and to identify the difference between them.

Keywords: isozymes, *Tephrosia*, phylogenetic analysis, peroxidase and esterases

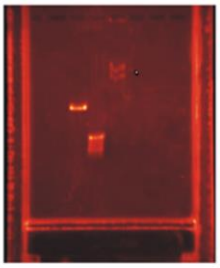
1. Introduction
In recent years, uses of isozyme data for plant taxonomic purposes have increased rapidly. This method offers a very powerful tool in studying lower hierarchies of plant taxa such as the species, sub-species or population level (Reisenberg *et al*., 1988; Burden *et al*., 1980; Brown, 1990). In order to ascertain the level of generic variation within and/or between populations, isozymes and allozymes can be used as main sources of data, because an enzyme marker, which is separated by electrophoresis, furnished a simple means for rapid partitioning of the variability within and between populations at the gene level. Isozyme data is particularly useful if the morphological characters of species appear to overlap. At the present time, there is no doubt that isozyme data provides a powerful tool in the era of molecular taxonomy. It is likely that by the next decade the use of isozyme data in the work of taxonomist will be intensively adopted. Molecular biology techniques will use data both from DNA sequence and isozyme analysis for research purposes in plant taxonomy. A new experimental method using SDS-PAGE will be used to explore the isozyme data. Genetic markers generally have contributed to the study of plant biology by providing methods for detecting genetic differences among individuals. There are some important ecological topics which often use isozymes as powerful markers Genetic relatedness within and among populations, often with relations to geographic structure. Genetic diversity in clone plant species. Forestry and agriculture. It is often necessary to test paternity or chromosomal locations and mapping. Selection and linkage in plant populations and their evolutional consequences. Conservation biology is a rapidly rising field because of effective molecular tools. Other studies like Seed bank populations, environmental changes and habitat heterogeneity, combination with cytological studies and other methods, plant pathology, germ plasm collections, mycorrhizal genetic variation and phytopathology.

$$Rf\ Value = \frac{Distance\ migrated\ by\ the\ enzyme\ band\ from\ the\ cathodal\ edge}{Distance\ migrated\ by\ the\ dye\ marker}$$

2. Material and Methods
1. All plants are collected from Near around places of Solapur district. The collected plants are grown under natural daylight conditions in Botanical Garden of D.B.F Dayanand College Of Arts & Science, Solapur.
2. From well grown plantlets young leaves are collected and centrifugation was carried out with various salt for isolation of DNA.
3. Isolated DNA was taken for molecular weight determination by using standard markers.
4. Protein analysis was carried out by Biuret method
5. Centrifugation was carried out to isolate isozymes, isolated isozymes were carried out to perform SDS-PAGE.
6. After completion of SDS-PAGE isozymes were stained by using different staining agents.
7. Staining Procedures:
   a. **Peroxidase:** 0.0125 grams of o-Dianisidine, dissolved in 2.5 ml of acetone, then add 50ml of 0.2 id acetate buffer pH 4.5 and 2 drops of H2O2.
   b. **Esterase:** 0.0125 grams of 1-naphthyl acetate dissolved in 2.5ml of acetone, then add 50ml of 0.2M phosphate buffer pH 6.5 and 0.0125 grams of fast blue BE salt.

Peroxidase Isozyme Pattern for *T.Purpurea, T.Pentaphylla.*

Peroxidase Isozyme Pattern for *T.Purpurea, T.Pentaphylla.*



Mol. Wt. determination of *T.Purpurea, T.Villosa, T.Pentaphylla.*

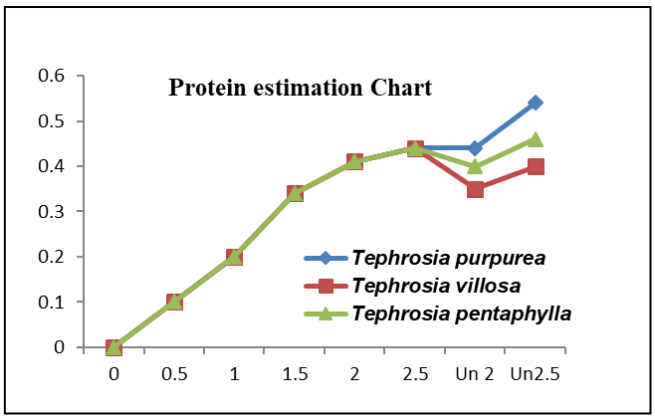
Protein Band Pattern of *T.Purpurea, T.Villosa, T.Pentaphylla.*

3. Observation

Table 1

Standard BSA (ml)	D/W (ml)	Biuret reagent (ml)	Incubate at room temp. for 30 min.	O.D at 530 nm.		
				Tephrosia purpurea	*Tephrosia villosa*	*Tephrosia pentaphylla*
0.0	2.5	2.5		0.0	0.0	0.0
0.5	2.0	2.5		0.10	0.10	0.10
1.0	1.5	2.5		0.20	0.20	0.20
1.5	1.0	2.5		0.34	0.34	0.34
2.0	0.5	2.5		0.41	0.41	0.41
2.5	0.0	2.5		0.44	0.44	0.44
Uk	0.5	2.5		0.44	0.35	0.40
Uk	0.0	2.5		0.54	0.40	0.46

Concentration of std. protein is 5mg/ml.



4. Result and Discussion

The result shows that the DNA molecular weight of *Tephrosia purpurea, Tephrosia villosa and Tephrosia pentaphylla* are 350bp, 400bp and 550bp respectively, Protein concentration found in species *Tephrosia purpurea* is 0.36mg/ml, *Tephrosia villosa* 0.40 mg/ml and *Tephrosia pentaphylla* is 0.46mg/ml. basically two main enzyme systems are present in Genus *Tephrosia* viz. peroxidase and esterase. While at the time of performing the work it was found that isozyme peroxidase present in *Tephrosia villosa* and *Tephrosia pentaphylla*, esterase isozyme was present in *Tephrosia purpurea* and *Tephrosia pentaphylla* having the Rf values 0.54 and 0.08 respectively. This useful investigation about the molecular weight of DNA, Protein concentration & isozyme pattern of *Tephrosia* is useful in many ways to the taxonomist to find out the common ancestors of these species to conserve and propagate that Species. As these species are economically important and source of high protein very useful diet for grazing animals. This study will further help to scientist working on taxonomy.

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