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Role of isozymes in systematic study of *Tephrosia purpurea*, *Tephrosia villosa* and *Tephrosia spinosa*

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ABSTRACT

Selected isozymes were investigated in the plants *Tephrosia purpurea* (L.)Pers, *Tephrosia villosa* (L.)Pers. and *Tephrosia spinosa* (L.f.)Pers. The standard technique of vertical poly acrylamide gel electrophoresis was employed. The gels were stained for different enzymes systems viz. esterase, peroxidase, acid phosphatase, alkaline phosphatase and polyphenol oxidase. The isozymes were fixed by using the standard procedure, the number, position and polymorphisms were recorded. The present study produced the biochemical markers for these three important weedy medicinal plants. The similarity index and variations between these three species are reported with reference to enzymes esterase, peroxidase, acid phosphatase, alkaline phosphatase and polyphenol oxidase.

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KEYWORDS

Bio-chemical marker;
Isozymes;
Tephrosia purpurea;
Tephrosia villosa;
Tephrosia spinosa;
Zymogram.

INTRODUCTION

For any living organism, identity is very important in order to distinguish itself from other organisms within the population and other populations. In plant taxonomy, during this molecular era also, the morphological characters play a vital role in plant systematic study and used as a tool for the classification of a taxon. In recent times, in addition to morphological markers, anatomical, cytological, biochemical and molecular markers are also being used to classify the organisms. The morphological and anatomical markers have several disadvantages when used as markers in botanical studies, e.g. they can not 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. But through biochemical analysis or specifically using isoenzymes, species can be easily distinguished^[2]. Since 1930, electrophoresis joined with the zymogram technique has been the tool of choice for studies of heritable variation by geneticists, systematists and population biologists. Isoenzymes are a powerful tool for gene variability within and between the populations of plants and animals, 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^[17,1,6,85,12]. Isozymes are

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useful as genetic and biochemical markers and also as good estimators of genetic variability in plant populations. 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^[10]. Isozyme markers have been successfully used in several crop improvement programmes^[11,17,14,12,15,16,13]. Based on the available background the present study was intended to characterize the medicinally important weedy plants *Tephrosia pupurea* (Linn). Pers., *Tephrosia villosa* (L.)Pers. (Toxins) and *Tephrosia spinosa* (L.f). Pers. The leaves and leaf buds of *T. purpurea* are ground and mixed with salt, niu (coconut, *Cocos nucifera*), as a topical treatment for cuts; also for itchy skin. It cures liver and spleen disorders, cough, fever, treats dyspepsia and colic in mixtures for impotency. Parts of the plant contain tephrosin, which stuns fish but not mammals, used in tide pools to poison and capture fish. *T. villosa* leaf juices are used for dropsy, root used in tooth paste^[7]. In this study, we investigated the genetic control of the isozymes of the enzymes esterase, peroxidase, acid phosphatase, alkaline phosphatase and polyphenol oxidase, in order to illustrate the use of enzyme polymorphism for the identification of *T. pupurea*, *T. villosa* and *T. spinosa*. The results obtained in the present investigation are expected to be useful for researchers dealing with plant systematics, pharmaceuticals and for plant breeders.

Abbreviations

EST – Esterase, PRX - Peroxidase, ACP - Acid phosphatase, ALP - alkaline Phosphatase, PPO - Polyphenol oxidase.

MATERIALS AND METHODS

Tephrosia pupurea(Linn). Pers., *Tephrosia villosa* (L.)Pers.(Toxins) and *Tephrosia spinosa* (L.f). Pers. were used as the experimental materials. 500 to 1000mg of freshly harvested young leaves were taken and homogenized with 3.5ml of ice-cold homogenizing buffer in a pre-chilled pestle and mortar. For peroxidase, the young shoots were homogenized with 0.1M phosphate buffer (pH 7.0) and centrifuged at 12,000rpm for 10min. For esterase, the young leaves were collected and

ground with pre-chilled isolation buffer(0.1M phosphate buffer pH 9.2) and centrifuged at 12,000rpm for 10min. For acid and alkaline phosphatase the young leaves were harvested and homogenized in a mortar and pestle with citrate buffer and centrifuged at 20,000 rpm for 10min. The supernatant was subjected to electrophoresis as described by Sadasivam and Manickam^[9] on PAGE. For the detection of isozymes on the gels, the staining solution, were prepared as per Sadasivam and Manickam^[9]. After the electrophoresis, the gels were incubated in the staining solution for few minutes under dark condition till the clear bands appeared. The gels were fixed with 7% acetic acid solution for 30min, washed with distilled water and photographed using the gel documentation system, Genei Bangalore, India.

RESULTS AND DISCUSSION

Acid phosphatase (ACP)

Multiple regions of activity were obtained for this enzyme system ACP 1 to 7. Region 1 contained two bands, ACP 1^{1,2}. ACP 1¹ was restricted to *Tephrosia villosa*, ACP 1² for *Tephrosia spinosa*. The *Tephrosia pupurea* did not show any band in this region. Region 2 contained a single band, very specific to *Tephrosia villosa* only (ACP 2¹); other two species not have shown any band in this region. Region 3 contained bands in three different positions; *Tephrosia spinosa* has not shown any band in this region. *Tephrosia pupurea* showed in two positions ACP 3^{1,3}, *Tephrosia villosa* contained a single band ACP 3². Region 4 contained bands in two different positions. In this region also *Tephrosia spinosa* has not shown any band. *Tephrosia villosa* showed a single band ACP 4¹ and *Tephrosia pupurea* also obtained a single band but in other location (ACP 4²). Region 5, showed two different positions in the banding profile, ACP 5¹ observed in *Tephrosia pupurea* and ACP 5² obtained in *Tephrosia villosa*, *Tephrosia pupurea* has not shown any band. Region 6, in *Tephrosia villosa* has not shown any band, *Tephrosia spinosa* and *Tephrosia pupurea* showed the banding profile in two different position each species occupied one positions (ACP 6¹-*Tephrosia spinosa* and ACP 6² - *Tephrosia pupurea*). Region 7 showed only one position which was restricted to *Tephrosia pupurea* (ACP 7¹), and the

other species did not show any profiles in this region (Figure 1A).

Peroxidase (PRX)

Three zones of activity (Region) were observed in this enzyme system. Region one contained a single band (PRX 1¹), whose position did not vary in any of the species used in this system. Similar to region one, Region two contained a single band (PRX 2¹), whose position did not vary in any of the species used in this system and it showed the similarities. The third one showed only one band (PRX 3¹) with restriction *Tephrosia pupurea* (Figure 1 D).

Alkaline phosphatase (ALP)

Three zones of activity were obtained. In *Tephrosia pupurea*, the first zone of activity was very faint and disappeared in a very short time. In *Tephrosia villosa* the band was dark in colour (ALP 1¹). In *Tephrosia spinosa*, the first zone activity was absent. The second zone of activity was found only in *Tephrosia pupurea* (ALP 2¹). The third zone of activity contained three bands (ALP 3¹⁻³), the ALP 3² was observed only in *Tephrosia pupurea*. The ALP 3³ was obtained in *Tephrosia spinosa* and *Tephrosia villosa*. The ALP 3¹ was restricted to *Tephrosia villosa* in this enzyme system (Figure 1 C). Esterase (EST)

There were multiple regions of activity for this enzyme system (EST 1-7). Region 1, contained band in three different positions, the first band was restricted to *Tephrosia pupurea* (EST 1¹), second one was restricted to *Tephrosia spinosa* and *Tephrosia villosa* (EST 1²). The banding profile of *Tephrosia pupurea* showed the variation in the third position also (EST 1³). Region 2 showed a single band, *Tephrosia villosa* and *Tephrosia spinosa* showed its presence and similarity (EST 2¹). Region 3 contained bands in two different positions; *Tephrosia spinosa* has not shown the band in this region, while other two species also showed the variation in the banding position (EST 3^{1,2}). The EST 3¹ was specific to *Tephrosia pupurea* and EST 3² to *Tephrosia villosa*. The EST 3⁶ was poorly stained; attempt to improve the resolution and staining was not successful. Region 4 contained bands in two different positions and the profiles were restricted to *Tephrosia pupurea* (EST 4^{1,2}). The other two species were not having any band-

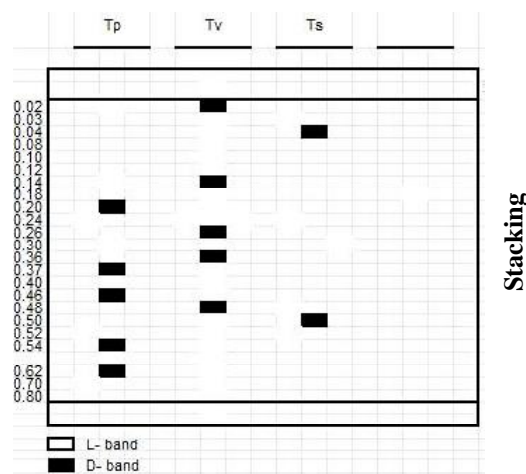


Figure 1(A): Zymogram of ACID phosphatase banding patterns of tephrosia spp.

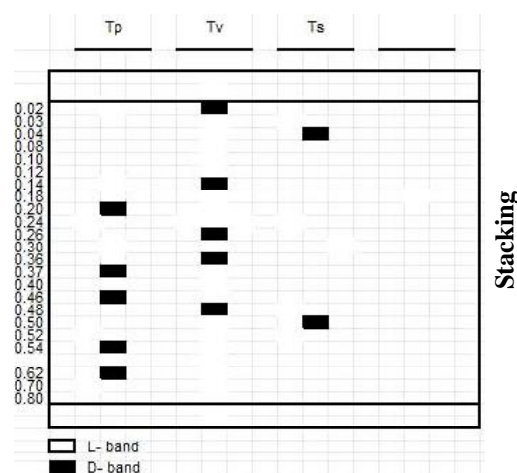


Figure 1(B): Zymogram of ISO esterase banding patterns of tephrosia spp.

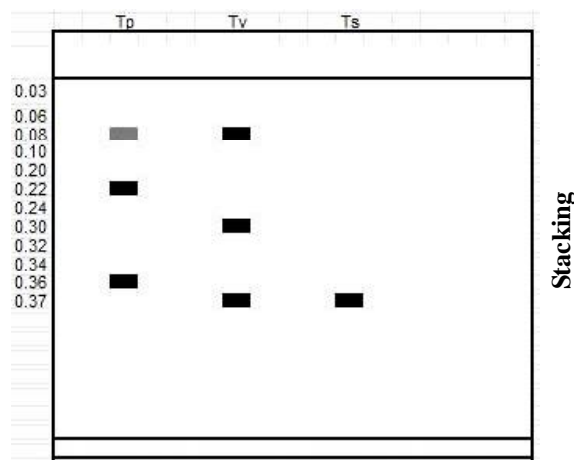


Figure 1(C): Zymogram of alkaline phosphatase banding patterns of tephrosia spp.

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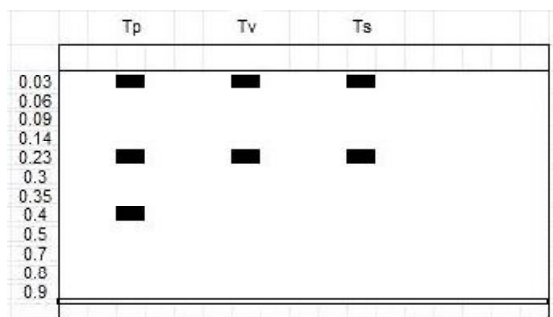


Figure 1(D): Zymogram of peroxidase banding patterns of tephrosia spp.

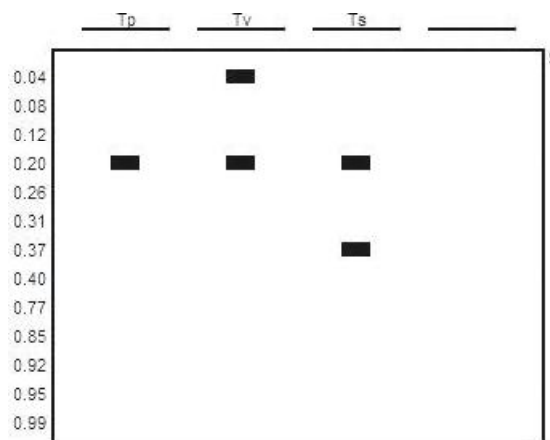


Figure 1(D): Zymogram of poly phenol oxidase banding patterns of tephrosia spp.

ing profile in this region. Similar to region 4, region 5 also showed a single band restricted to Tephrosia pupurea, other two species failed to express in this region (EST 5¹). Region 6 showed three bands in different positions (EST 6¹⁻³) but the (EST 6¹⁻³) were specific to Tephrosia villosa and Tephrosia spinosa, EST 6² was specific to Tephrosia pupurea. Region 7 found bands in two different positions (EST 7^{1,2}). The region 7 showed very clearly the similarity between the Tephrosia villosa and Tephrosia spinosa and variation from Tephrosia pupurea. The EST 7¹ was very specific to Tephrosia villosa and Tephrosia spinosa, the EST 7² to Tephrosia pupurea (Figure 1B).

Polyphenol Oxidase (PPO)

Three zones of activity were obtained in this enzyme system. The first zone of activity was faint and disappeared with very short time (PPO 1¹). The second zone of activity contained a single band, the band was ob-

served for all accessions used in this study (PPO 2¹). The third zone of activity contained a single band that was specific to Tephrosia spinosa (PPO 3¹) (Fig. 1 E).

Regarding the enzymes esterase, peroxidase, acid phosphatase, alkaline phosphatase and polyphenol oxidase, the similarity and variation between Tephrosia pupurea, Tephrosia villosa and Tephrosia spinosa are reported (Figure 1 A to E). The present study revealed that, the selected three species were easily separable isozymically. According to Hamrick and Godt^[3] Isozymes are practical, useful genetic and biochemical markers as well as good estimators of genetic variability in plant populations. In the present study also isozymes were used as biochemical marker to distinguish the selected plant species and coincide with Hamrick and Godt^[3] observations. The presence or absence of chemical constituent has been found useful in the placement of the plant in taxonomic categories. The presence and absence of the isozyme can be revealed by biochemical methods. Isozymic differentiation is one such important and powerful procedure which has often employed for this purpose. Each isozyme has a specific role in the metabolic pathway and functions in harmony with other enzymes within the organizational framework of cells. Isozymes often exhibit tissue or cell specificity. Isozymic variation has been chosen here to reveal the diversity existing at molecular level in Tephrosia pupurea, Tephrosia villosa and Tephrosia spinosa. The present study confirmed the role of isozymes in species diversity variation and similarity between the selected species. Each zone is occupied by a particular isozyme in the form of band and is representative of the expression of a particular gene locus coding for that isozyme. In certain enzyme system, in a particular zone more than one distinct band are resolved. These bands could represent allelic isozymes, coded by different alleles of the same gene at locus and thus occupy that particular zone on the gel. In the present study also the similar kind of banding profiles are observed in all enzyme systems indicating the presence of multiple alleles. Isozymes such as esterase and peroxidase have been utilized to trace the genetic lineage of various rice varieties^[4,12,13,15,16], sugarcane cultivars^[5], pulses^[14], medicinal plants^[6,8]. Similarly in the present study also, the isozymes are used as biochemical marker for the systematic study of Tephrosia species. Unique

banding profiles of esterase, peroxidase, acid phosphatase, alkaline phosphatase and polyphenol oxidase were observed in *Tephrosia pupurea*, *Tephrosia villosa* and *Tephrosia spinosa*, which represent the fingerprint of that particular species. Such finger printing is useful in differentiating the species and act as biochemical markers for these species in plant systematic studies. Molecular marker and isozyme sequencing are considered excellent for population structure analysis but the data obtained through isozyme is relatively inexpensive compared to DNA. In addition, a large number of samples can be processed with far less training and time per sample, whereas DNA analysis requires more time and sophisticated instruments. Furthermore, in most cases the new DNA - based markers provide the same type of information as isozymes. Electrophoretic studies indicated that *Tephrosia villosa* and *Tephrosia spinosa* had more alleles in common with each other than they had with *Tephrosia pupurea*. These results indicate that *Tephrosia pupurea* is electrophoretically distinct from *Tephrosia villosa* and *Tephrosia spinosa*.

REFERENCES

- [1] D.Crawford; Syst.Bot., **10**, 405 (1985).
- [2] L.D.Gotllieb; Biochem.Genetics., **9(1)**, 97-107 (1973).
- [3] J.L.Hamrick, M.J.W.Godt; Crop.Science, **37(1)**, 26-30 (1997).
- [4] S.Hiraga, K.Yamamoto, H.Ito, H.Matsui et al.; FEBS Letts., **471**, 245-250 (2000).
- [5] B.R.Manjunatha, S.Virupakshi, G.R.Naik; Curr.Sci., **85**, 1347-1349 (2003).
- [6] A.N.Onus, B.Pickergill; Turk.J.Bot., **24**, 311-318 (2000).
- [7] N.Rajakaruna, S.C.Harris, G.H.N.Tower; Pharmaceutical Biology., **40(3)**, 235-244 (2002).
- [8] K.K.Sabu, P.Padmash, S.Seeni; J.Med.Arom. Plant.Sci., **23**, 637-647 (2001).
- [9] S.Sadasivam, A.Manickam; 'Biochemical methods for Agricultural Science', Chapter 4.2 Wiley Eastern Ltd. and Tamil Nadu Agricultural University, Coimbatore, India, 139 (1992).
- [10] J.G.Scandalios; 'Genes, Isozymes and Evolution', In, L.M.Clement (ed.); Isozymes IV. Genetics and Evolution, Academic Press, New York, 1-7 (1975).
- [11] H.Smila, M.Johnson, M.Rajasekarapandian; Ind.J.Biotechnology., **6**, 91-99 (2007).
- [12] K.N.Srivatatava, M.Rai, R.S.Tyagi, G.Kaur; Ind.J.Plant.Physiol., **7**, 227-233 (2002).
- [13] H.S.Suh, Y.I.Sata, H.Morishma; Thero.Appl.Genet., **94**, 316-321 (1997).
- [14] W.Tadesse, E.Bekele; Lathyrus Lathyrism Newsletter, **2**, 43 -46 (2001).
- [15] H.Yanghong, S.Xinli, W.Xiangkun; Agric.Arch., **4**, 2-8 (1984).
- [16] W.J.Yu; Zuower Pinshong Ziyuan., **1**, 35-36 (1987).
- [17] M.Zeidler; Acta,Univ.Palacki.Olomuc.Fac.Rer.Nat. Biol., **38**, 7-16 (2000).