ASSESSMENT OF GENETIC DIVERSITY OF SELECTED *TEPHROSIA* SPECIES FROM TIRUNELVELI DISTRICT OF TAMIL NADU

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Abstract: The genus *Tephrosia* is one of the important genera of the family fabaceae and having 400 species throughout the world. Most of the species of the genera are morphologically alike but differ only in few characters. In the present investigation, we have analyzed the genetic variability of selected five species (*T. purpurea*, *T. villosa*, *T. spinosa*, *T. maxima* and *T. procumbens*) using ISSR (Inter Simple Sequence Repeat) markers. The species were collected from in and around of Tirunelveli districts of Tamil Nadu. There are ten ISSR primers were screened for the study out of which five reproducible primers were used for further analysis. The five primers were produced 29 scorable bands with five *Tephrosia* species. The number of polymorphic loci was 13 and polymorphic percentage was 56.52%. The genetic distance between the *Tephrosia* species was ranged from 0.4229 to 0.8023 and genetic diversity was ranged from 0.4483 to 0.6552. The mean value of overall observed and effective number of alleles was about 1.9310 and 1.6410. Nei's overall genetic diversity and Shannon information index was 0.3697 and 0.5433. The similarity matrix obtained in the present study was used to construct a dendrogram with the UPGMA method and resulted in their distant clustering in the dendrogram. The results indicated that the similarity and dissimilarity between selected *Tephrosia* species.

Keywords: Genetic diversity, Molecular marker, ISSR, *Tephrosia*, Tirunelveli.

I. INTRODUCTION

The genus *Tephrosia* comes under the family fabaceae. It is a pantropical taxa with about 400 species chiefly in Asia, Africa, Australia and America (Gillett, 1971). In India about twenty-four species of *Tephrosia* were recorded (Gamble and Fischer, 1918). Mostly *Tephrosia* genus plants are herbs or shrubs. The plants are prostate or erect herbs or in the form of soft or woody shrubs (Hacker, 1990). Most of the plants from this genus have been used traditionally for the treatments like rheumatic pains, syphilis, dropsy, stomach ache, diarrhea, asthma, abortifacient, respiratory disorderrs, laxative, diuretic, and inflammation etc. (Qureshi *et al*., 2010; Dzenda *et al*., 2007).

Traditional classification of the various subgenera, species and subspecies is based primarily on morphological attributes. However, these traits may not be significantly distinct and usually require growing plants to maturity prior to identification. Moreover, morphological characters may be unstable due to environmental influences. Over the years, the methods for detecting and assessing genetic diversity have extended from analysis of discrete morphological traits to biochemical and molecular traits. Several DNA marker systems are now common use in diversity studies of plants. It is less time consuming and easy to handle in comparison with morphological and biochemical markers. It helps to display the closeness of species and hybrids accurately (Soller and Beckmann, 1983). Many molecular markers like RAPD, ISSR, AFLP and SSR are currently used in genetic diversity study of plants, depending upon the nature of the study the suitable markers will be selected (Vos *et al*., 1995; Zietkiewicz *et al*., 1994; Becker and Heun, 1994).

ISSR marker is one of the best molecular markers used in DNA fingerprinting studies of plants due to a lot reasons. ISSR based markers have service in the fields such as genetics, taxonomy, physiology, embryology etc. and also have broad applications in pharmacognostic characterization of

medicinal plants (Kurane *et al*., 2009). Therefore, studies of population genetic diversity and the structure of population within and between species may not only illustrate the evolutionary process and mechanism but also provide information useful for biological conservation (Notter, 1999). So the present investigation is framed to explore the inter-specific genetic relationship between five *Tephrosia* species using ISSR marke.

II. MATERIALS & METHODS

The following Fabaceae members were selected for the present study. The selected plant materials were identified and collected from in and around Tirunelveli regions of Tamil Nadu. The healthy, young and fresh leaves were collected from their natural habitats and stored in deep freezer for the DNA isolation and ISSR-PCR analysis. The experimental materials selected for the present study are *Tephrosia purpurea, Tephrosia villosa, Tephrosia maxima, Tephrosia spinosa* and *Tephrosia procumbens b*elonging to the family fabaceae.

Isolation of Genomic DNA

The genomic DNA was extracted using the standard protocol of Doyle and Doyle () [21]with slight modification. The quality and quantity of the DNA was check with 1% agarose gel electrophoresis and UV- Visible spectrophotometer optical density respectively and the isolated genomic DNA stored in deep freezer for further studies.

ISSR PCR Analysis

The amplification of PCR mixture was done with 20 µl reaction volume has 2x DyNAzyme II PCR Master Mix 10 µl. Among ten primers, the following five primers UBC810 (GAG AGA GAG AGA GAG AT), UBC814 (CTC TCT CTC TCT CTC TA), UBC823 (TCT CTC TCT CTC TCT CC), UBC827 (ACA CAC ACA CAC ACA CG) and UBC891 (HVH TGT GTG TGT GTG TG) were selected for final experiment based on the ability of producing reproducible bands. The PCR reaction mixture was mixed gently in spinwin and 35 cycles of reaction was performed in a PCR (Applied Bio systems) with the amplification profile; of 95^0C - 5.00 min for initial denaturation, 94^0C -0.45 min for denaturation, 42^0C - 1.00 min for annealing, 72^0C - 1.30 min for extension, 72^0C - 10.00 min for final extension and 4^0C -∞ followed by cooling at 4^0C. The final product of the PCR was checked in more than one percent agarose gels. The resulting gel was visualized and documented in Bio-Rad Gel documentation system.

Interpretation of Data

The presence and absence of clear, dense and visible bands in the gels of different populations were noted (Williams *et al.*, 1990). The data was analysed with the help of Pop gene package version 1.31and the dendrogram was constructed with MEGA software. Nei and Li (1979) method was used for the calculation of individual population similarity index.

III. RESULTS & DISCUSSION

Recent advances in molecular biology provide novel tools for addressing evolutionary, ecological and taxonomic research questions. Variation in DNA sequence can be observed with a level of accuracy and throughput that was previously impossible. The bulk of variation at the nucleotide level is often not visible at the phenotypic level. This DNA variation is frequently exploited in molecular genetic marker systems, and the application of molecular markers to advance research and commercial activities is now well established (Gupta *et al.*, 2005). DNA based markers have many advantages over phenotypic markers in that they are highly heritable, relatively easy to assay, and are not affected by the environment (Newbury and Ford-Lioyd, 1993). Electrophoresis analysis along with field evaluation gives additional information to taxonomy and should not be disassociated from morphological, anatomical and cytological observation (De Vries, 1996).

In the present study ISSR marker was used to assess the genetic diversity among five selected *Tephrosia* species. There are five ISSR primers were used for the analysis and produced 29 scorable bands. The number of bands produced by each primer displayed in figure 1. The number of polymorphic loci is 13 and the percentage of polymorphic loci is 56.52. The genetic distance between the *Tephrosia* species ranged from 0.4229 to 0.8023 and the genetic identity is ranged from 0.4483 to 0.6552 (Table 1). The genetic variation statistics were displayed in Table 2. The mean value of overall observed and

effective number of alleles is about 1.9310 and 1.6410. Nei's overall genetic diversity and Shannon information index is 0.3697 and 0.5433 (Table 1). The similarity matrix obtained in the present study was used to construct a dendrogram with the UPGMA method and resulted in their distant clustering in the dendrogram (Figure 2). The length between populations mentioned in the branches of dendrogram and in Table 3.

The dendrogram was constructed based on the Nei's (1978) Genetic distance: Method = UPGMA--Modified from NEIGHBOR procedure of PHYLIP Version 3.5. The dendrogram sowed the genetic relationship between the selected five *Tephrosia* species. Among the five selected taxa *T. villosa* and *T. spinosa* showed more relationship compared to other three taxa. *T. procumbens* separated into new cluster and sowed more similarity with *T. villosa* and *T. spinosa* and less dissimilarity with *T. maxima*. *Tephrosia maxima* also separated into new cluster. *T. purpurea* showed more dissimilarity with other four taxa and separated into new cluster. Among the five taxa more dissimilarity observed in *T. purpurea* and more similarity observed in between *T. villosa* and *T. spinosa.* The result reflected the distribution of *T. purpurea* was common in all the places of Tirunelveli district, all the other four species restricted to some places.

ISSR markers are proving very useful for correct botanical identification in plant taxonomy. They can clearly distinguish intra and inter species variation. There are several studies in which these markers are used for species or cultivar identification. The level of polymorphism in tomato (*Lycopersicon esculentum*) was studied using ISSR-PCR. Five tomato species: *Lycopersicon esculentum*, *Lycopersicon pennellii*, *Lycopersicon cheesmanii*, *Lycopersicon humboldtii*, *Lycopersicon hirsutum* and two *Lycopersicon esculentum* substitution lines IL 6-3 and WSL 6 were analyzed. ISSR-PCR was performed with fourteen primers. Nine of these fourteen primers were individually able to distinguish all tomato species (Yu *et al.*, 2003). ISSR analysis was performed in eight cultivars of eggplant (*Solanum melongena*) and 12 accessions in eight related *Solanum* species to evaluate the applicability of this analysis for assessing the phylogenetic relationships and identifying cultivars. A total of 552 polymorphic amplified bands were obtained from 34 of the 100 primers tested and the percentage of polymorphisms was 99.1 % (Isshiki *et al.*, 2008).

Table 1: Nei's genetic identity mentioned in above diagonal and Genetic distance in below diagonal

Species	*T. purpurea*	*T. villosa*	*T. maxima*	*T. spinosa*	*T. procumbens*
T. purpurea	****	0.4828	0.4483	0.4828	0.4828
T. villosa	0.7282	****	0.5517	0.6552	0.5172
T. maxima	0.8023	0.5947	****	0.5517	0.5517
T. spinosa	0.7282	0.4229	0.5947	****	0.6552
T. procumbens	0.7282	0.6592	0.5947	0.4229	****

Table 2: Summary of genetic variation for all Loci

Statistic parameters	Mean value	Standard value
Observed number of alleles (a)	1.9310	0.2579
Effective number of alleles (ne)	1.6410	0.2843
Nei's(1973) gene diversity (h)	0.3697	0.1290
Shannon's information index (I)	0.5433	0.1727

Table 3: Length inbetween populations

Between	And	Length
4	pop1	37.33827
4	3	7.60292
3	2	2.68279
2	1	5.90972

1	pop2	21.14284
1	pop4	21.14284
2	pop5	27.05256
3	pop3	29.73536

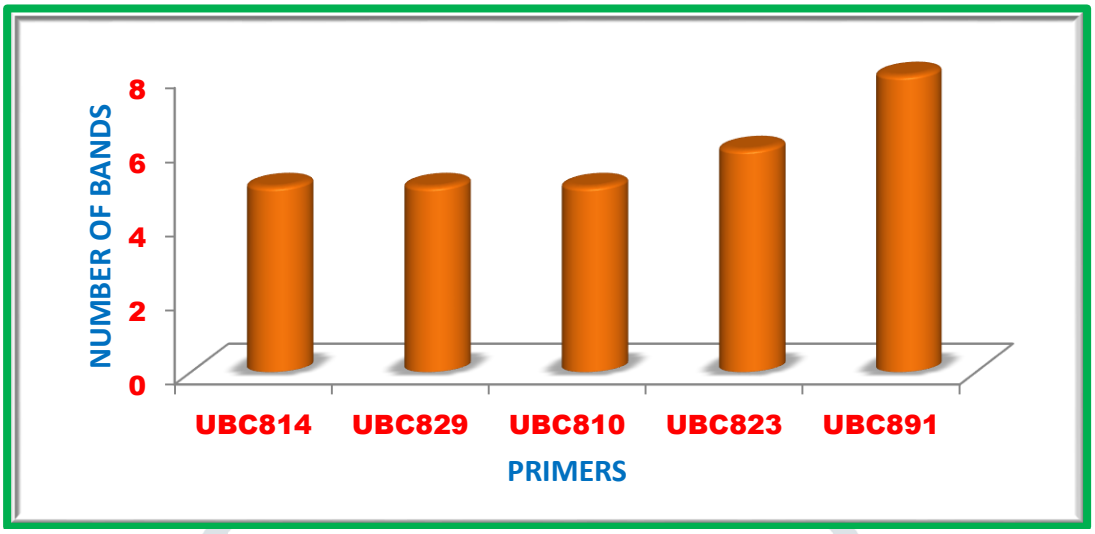

Figure 1: Number of Bands produced by each primer

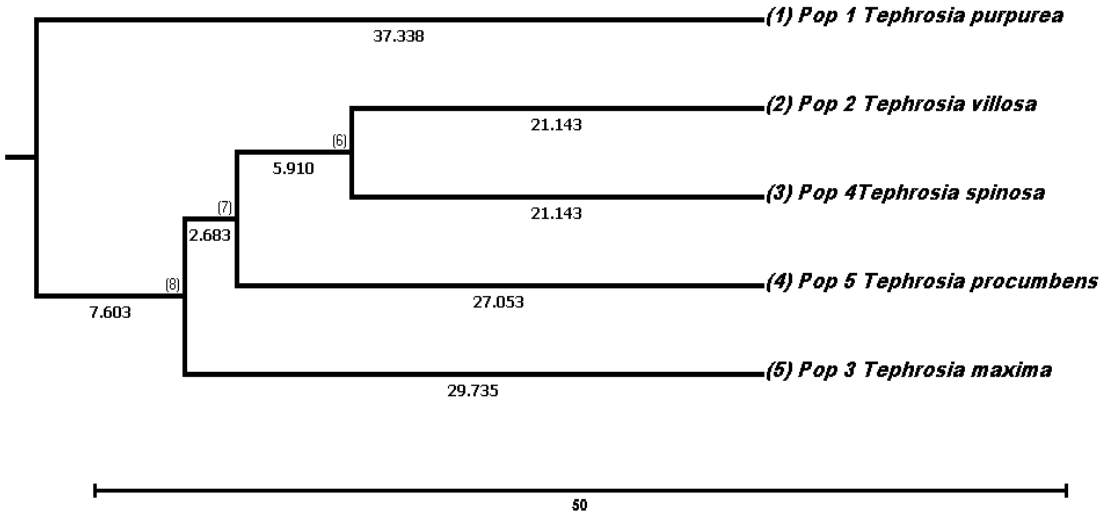

Figure 2: UPGMA Dendrogram of *Tephrosia* species

IV. CONCLUSION

In the present investigation the genetic relationship between the selected species of *Tephrosia* was studied with molecular marker ISSR. The results will helpful to know the degree of closeness and degree of difference between the five selected *Tephrosia* species. The morphological marker or biochemical marker based classification of plants may be affected by various environmental factors, but the DNA based molecular marker will overcome that problem and help to authentication of plants in plant taxonomy.

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VI. REFERENCES

Becker, J and Heun, M. 1994. Barley microsatellites: allele variation and mapping, Plant Molecular Biology, 27(4): 835-845.

De-Vries, IM. 1996. Characterization and identification of *Lactuata sativa* cultivars and wild relatives with SDS-electrophoresis (*Lectuca sect. Lectuca*, Compositae), GRACE. Genet. Res. Crop Eval., 43: 193-202.

Doyle, J. J., & Doyle, J. L. (1987). A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue. Phytochemical Bulletin, 19(1), 11-15.

Dzenda, T., Ayo, JO., Adelaiye, AB., Adaudi, AO and Ibrahim, ND. 2007. Preliminary Investigation Into the acute oral toxicity of *Tephrosia vogelii* Leaves In Mice, Nigerian Veterinary Journal, 28: 47-52.

Gamble, JS and Fischer, CEC. 1918. Flora of Presidency of Madras; Botanical Survey of India - Howrah – India.

Gillett, JB. 1971. Flora of Tropical East Africa Leguminosae Part 3 Sub family Papilionoideae (1) Crown Agents, London- U.K.

Gupta, PK., Rustgi, S and Kulwal, PL. 2005. Linkage disequilibrium and association studies in higher plants: present status and future prospects. Plant Mol. Biol., 57:461–485.

Hacker, JB. 1990. A guide to herbaceous and shrub legumes of Queensland', University of Queensland Press, pp. 275.

Isshiki, S., Iwata, N and Khan, MR. 2008. ISSR variations in eggplant (*Solanum melongena* L.) and related *Solanum* species, Scientia Horticulturae, 117: 186-190.

Kurane, J., Shinde, V and Harsulkar, A. 2009. Application of ISSR marker in pharmacognosy: Current update, Phcog Rev., 3: 216-28.

Nei, M and Li, WH. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA, 76(10): 5269-5273.

Nei. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals, Genetics, 89: 583-590.

Newbury, HJ and Ford-Lioyd. 1993. The use of RAPD for assessing variation in plants, Plant growth reg., 12: 43-51.

Notter, DR. 1999. The importance of genetic diversity in livestock populations of the future, J Anim Sci., 77: 61-69.

Qureshi, R., Bhatti, GR and Memon, RA. 2010. Ethnomedicinal uses of herbs from northern part of NARA desert, Pakistan. Pak. J. Bot, 42: 839-851.

Soller, M and Beckmann, JS. 1983. Genetic polymorphism in varietal identification and genetic improvement, Theoretical and Applied Genetics, 67(1): 25-33.

Vos, PR, Hogers, R, Bleeker, M, Reijans, M, Lee T Van De, Hornes, M, Frijters, A, Pot, J, Peleman, J, Kuiper, M and Zabeau, M. 1995. AFLP: a new technique for fingerprinting, Nucleic Acids Research, 23(21): 4407-4414.

Williams, JG., Kubelik, AR., Livak, KJ., Rafalski and Tingey, SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res, 18(22): 6531-653.

Yu, M. Tikunov, LI., Khrustaleva, GI and Karlov. 2003. Application of ISSR markers in the genus *Lycopersicon*, Euphytica, 131: 71-80.

Zietkiewics, E., Rafalski, A and Labuda, D. 1994. Genome fingerprint by sequence repeat (SSR)-anchored polymerase chain reaction amplification, Genomics, 20: 176-183.