RESEARCH ARTICLE

Establishment of Phylogenetic relationship on the basis of SDS-PAGE among the *Dioscorea* species under section Opsophyton, Enantiophyllum and Lasiophyton from Melghat Tiger Reserve Maharashtra, India.

Gawande PA[1*], Deshmukh VP[1] , Choudhary US[1] and Thakare PV[2]

[1]*Department of Botany, Sant Gadge Baba Amravati University, Amravati-444602, Maharashtra.*

[2]*Department of Biotechnology, Sant Gadge Baba Amravati University, Amravati-444602, Maharashtra.*

**Corresponding author email : prashantagawande@yahoo.co.in*

Manuscript details:

Date of publication 18.10.2014

Available online on http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Gawande PA, Deshmukh VP, Choudhary US and Thakare PV (2014) Establishment of Phylogenetic relationship on the basis of SDS-PAGE among the *Dioscorea* species under section Opsophyton, Enantiophyllum and Lasiophyton from Melghat Tiger Reserve Maharashtra, India, *Int. J. of Life Sciences,* Special Issue A2: 77-80.

ABSTRACT

A crude protein extract when fractionated on a suitable gel medium produces a spectrum of bands which is diagnostic for the species. For each species at least 10 locations were selected. Protein samples were extracted in 50 mM Tri-HCl buffer (pH 8.3) to added 10 mM 2-Mercaptoehanol. Vertical polyacrylamide gel electrophoresis (SDS-PAGE) was carried out for separation of proteins, using crude extract. The 15% Gel caste was used for the separation of proteins. Genetic similarity (GS) between individuals was estimated and the matrix was subjected to Unweighted Pair Group Method for Arithmetic average analysis (UPGMA) to generate dendrogram using average linkage procedure. It was evident that these five species were in two clusters. *D. bulbifera*, *D. oppositifolia*, *D. hispida*, and *D. pentaphylla* formed a distinct cluster (cluster-I), while *D. belophylla* was completely out-grouped and formed cluster II. Although, *D. bulbifera* and *D. oppositifolia* were placed nearer to each other, they did not pair and hence, were placed in two different sub-clusters (sub-cluster I and II). In this cluster *D. hispida* and *D. oppositifolia* was grouped together and formed sub-cluster III. The *D. belophylla* was distantly related with remaining four species.

Keywords:*Dioscorea*, Dioscoreaceae, SDS-PAGE, Protein Profile, Phylogenetic analysis.

INTRODUCTION

The Melghat Tiger Reserve (MTR) is located on southern offset of the Satpura hill ranges in central India, called Gawilgarh hills in the Maharashtra. The family Dioscoreaceae is a natural group of tuber forming, tropical vines. The family is divided into two tribes: the Dioscoreae, including six genera all of which have unisexual flowers, and the Stenomeridae with three genera which produce hermaphroditic flowers (Smith, 1937).

The present investigator was very much impressed by the work carried out by Prain and Burkill in India as well as abroad. Burkill spent 58 years of his life on the study of world Dioscoreaceae (Burkill, 1960). Under the family Dioscoreaceae, the section Combilium includes *D. aculeata*, section Lasiophyton with *D. pentaphylla* and *D. triphylla*, Opsophyton with *D. bulbifera* and the last section Enantiophyllum represented by *D. wallichii*, *D. anguina*, *D. belophylla*, *D. glabra* and *D. alata* (Duthie, 1960). These sections were introduced earlier by Prain and Burkill (1936) who were the basic contributors on the taxonomy of Dioscoreaceae. The genus *Dioscorea* in the

forests of MTR is represented by five species namely, in the section Opsophyton includes species such as *D. bulbifera*; however section Enantiophyllum includes *D. oppositifolia* and *D. belophylla* and in the section Lasiophyton accomodate *D. hispida* and *D. pentaphylla* (Patel, 1968; Dhore and Joshi, 1988). Marked diversity was observed among the five species of the genus *Dioscorea* in MTR and also within individuals of the same species except *D. hispida*.

Protein electrophoresis has provided a new approach to the problems of species relationships (Johnson and Hall, 1965). SDS-PAGE technique was for the first introduced by Laemmli (1970). The proteins and enzymes are the important parameters in order to study molecular taxonomy (Anu and Peter, 2003). The proteins as primary gene products are good markers of genetic variations (Odeigah *et al*., 1999).

Dioscoreales have been the centre of attraction for plant systematists for many years. Salient features of *Dioscorea* viz. reticulate venation, nervation between primaries reticulate, ring vascular bundles, lateral position of the pistil, and second delayed cotyledon render the genus *Dioscorea* interesting for tracing possible phylogenetic relationship between Monocotyledons and Dicotyledons (Dhalgren *et al*., 1985; Brunnschweiler, 2004). By considering above character the present investigation intends to undertake in order to perform morphological character optimization, correlation of phenetic similarities with the genetic similarity and establishment of genetic relationship among genus *Dioscorea* from MTR. In relation to the above, the following parameters were undertaken to study genetic relationship between the five species of *Dioscorea* for to study inter-relationship based on morphology and protein profile.

MATERIALS AND METHODS

A total five individuals were randomly collected from each location on geographical and morphological basis as mentioned earlier. For each species at least 10 locations were selected. Tubers were cleaned with distilled water, pealed and cut into small strips. Small part measuring 1 gm from one location, thus samples from 10 locations were, dried at room temperature for 12 hours and transferred to oven at 55^{o}C for 24 hours, then grinded into fine powder in pestle and mortle. Tuber powder (200 mg) was taken from the above composite bulk and was homogenated at room temperature with 50 mM Tri-HCl buffer (pH 8.3) to added 10 mM 2-Mercaptoehanol, (Harvey and Boulter, 1983) and placed at 0^{o}C for 24 hours and then subjected to centrifugation at 13000 rpm for 15 min at 4^{o}C. The supernatant was transferred to 2 ml microcentrifuge tube containing 100 mg powder from bulk homogenate. The procedure was repeated twice. Protein electrophoresis was worked out according to the method of Laemmli (1970). Vertical polyacrylamide gel electrophoresis (SDS-PAGE) was carried out for separation of proteins, by using 15% Gel caste. Staining was performed by applying comassive brilliant blue stain R-250. Only the clear, unambiguous bands were considered for the preparation of unitary matrix by applying the method outlined by Nei and Li (1979). The phylogenetic analysis was carried out by using NTSYS-pc version 2.0 (Rohlf 1987) to generate similarity coefficient. The matrix was subjected to Unweighted Pair Group Method for Arithmetic average analysis (UPGMA) (Sokal and Micluner, 1958) to generate dendrogram using average linkage procedure.

RESULTS AND DISCUSSION

Two procedures were followed, one with 50 mM Tris-HCl buffer (pH 8.3) to which 10 mM β-mercaptoethnol and second with 50 mM Tris-Glycine buffer (pH 8.3) with 10 mM β-mercaptoethnol was added. Storage proteins were soluble maximally in Tris-HCl buffer because of its high ionic strength.

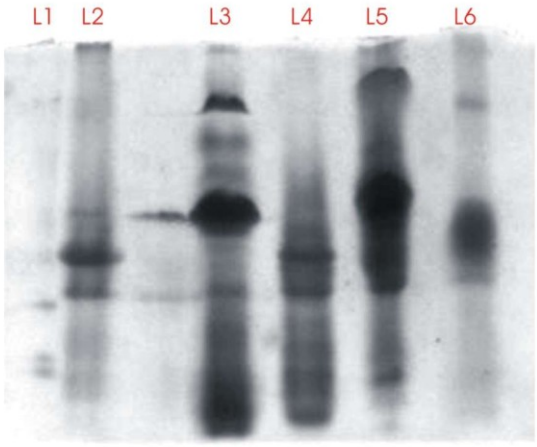

Fig. 1: SDS-PAGE Protein profile of *Dioscorea* species.

Table 1: Nei and Li similarity coefficient of SDS-PAGE

Species	*D. bulbifera*	*D. oppositifolia*	*D. hispida*	*D. pentaphylla*	*D. belophylla*
D. bulbifera	1				
D. oppositifolia	0.76	1			
D. hispida	0.66	0.75	1		
D. pentaphylla	0.75	0.82	0.84	1	
D. belophylla	0.60	0.54	0.46	0.42	1

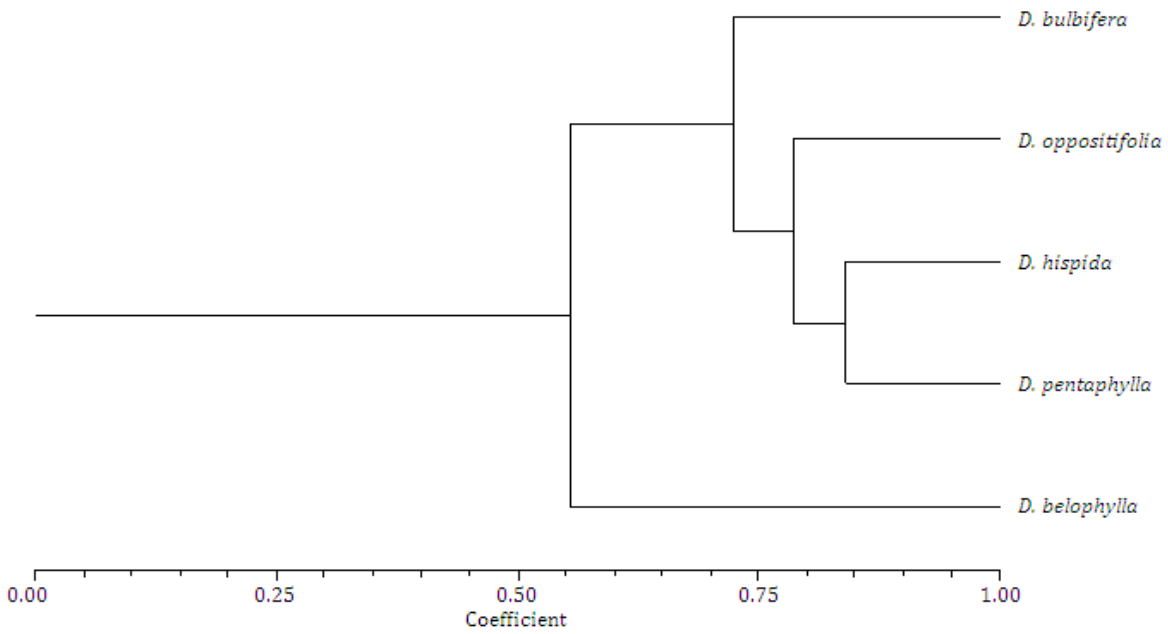

Fig. 2: Dendrogram showing genetic relationships between *Dioscorea* spp.

The five species of the genus *Dioscorea* were analyzed for proteins by 15% SDS-PAGE. Dormant tubers were taken for protein profile study. The tuber storage protein profile of five species of *Dioscorea* showed 11 stable bands, out of which 2 bands were monomorphic and 9 were polymorphic. The SDS-PAGE profile produced on an average 8 bands per species (fig. 1.). The highest similarity index was observed in *D. hispida-D. pentaphylla* (0.84) and the lowest in *D. pentaphylla-D. belophylla* (0.42). From similarity matrix (Table 1.) the dendrogram was constructed by using UPGMA method. It was evident that these five species were in two clusters. *D. bulbifera*, *D. oppositifolia*, *D. hispida*, and *D. pentaphylla* formed a distinct cluster (cluster-I), while *D. belophylla* was completely out-grouped and formed cluster II. Although, *D. bulbifera* and *D. oppositifolia* were placed nearer to each other, they did not pair and hence, were placed in two different sub-clusters (sub-cluster I and II). In this cluster *D. hispida* and *D. oppositifolia* was grouped together and formed sub-cluster III. The *D. belophylla* was distantly related with remaining four species (Fig.2).

The genus *Dioscorea* of MTR exhibited more diversity in morphology and protein profile as a biochemical marker used in present study was able to establish genetic relationship in the five species of MTR as well as for the taxonomic circumscription. Significant diversity was detected within the germplasm of *Dioscorea* spp. by isozyme pattern (Mignouna and Dansi, 2003). Cultivars of *Dioscorea cayenensis-rotundata* complex can be distinguished based on their morphological traits and/ or their isozyme pattern (Mignouna *et al*., 2002).

From dendrogram it was evident that these five species fell in to two clusters. The species *D. bulbifera*, *D. oppositifolia*, *D. hispida*, and *D. pentaphylla* formed a distinct cluster (cluster-I), while *D. belophylla* was

completely out-grouped and formed cluster II. The juvenile leaf of *D. oppositifolia* resembled with the mature leaf of *D. bulbifera*, moreover cormous head of *D. oppositifolia* apparently resembled with the tuber of *D. bulbifera*. In the dendrogram generated by SDS-PAGE these two species were placed nearer to each other but did not cluster, *D. bulbifera* of section Opsophyton was placed under sub-cluster I and *D. oppositifolia* of section Enantiophyllum under sub-cluster II. In the genus *Brachypodium*, *B. mexicanum* of section *Brachypodium* and *B. distachyon* of section Trachynia were clustered together in dendrogram generated on the basis of SDS-PAGE (Khan, 1992). In present investigation *D. belopylla* and *D. oppositifolia* of Enantiophyllum gets separated from each other. Similarly, cultivars of *D. rotundata* (white yam) and *D. cayenensis* (yellow yam) of section Enantiophyllum clearly separated from each other in dendrogram generated on the basis of isozymes (Mignouna *et al*., 2002). In the cluster I, *D. hispida* and *D. oppositifolia* of section Lasiophyton grouped together and formed sub-cluster III. The tripinnately compound leaf species *D. hispida* and *D. dumetorum* grouped together in the dendrogram generated on the basis of *rbc*L gene; *D. pentaphylla* and *D. hispida* placed nearer to each other on the dendrogram generated by *atp*B (Caddick *et al*., 2002). Morphological analysis brought *D. oppositifolia* and *D. hispida* under compound leaf clade (Wilkin *et al*., 2005). The *D. belophylla* of section Enantiophyllum was distantly related with remaining four species in the forest of MTR.

REFERENCES

Anu A, Peter KV (2003) Analysis of seed protein of 29 lines of *Capsicum annuum* L. by polyacrylamide gel electrophoresis. *Genet Resour Crop Evol* 50: 239-243.

Brunnschweiler J (2004) Structure and texture of yam (*Dioscorea* spp) and processed yam products. Ph.D thesis submitted for the degree of Doctor of natural sciences. University of Zurich

Burkill IH (1960) The organography and the evolution of Dioscoreaceae, the family of the yams. *J Linn Soc (Bot.*) 56 (367): 319-412 .

Caddick LR, Rudall PJ, Wilkin P, Hedderson TAJ, Chase MW (2002) Phylogenetics of Dioscoreales based on combined analyses of morphological and molecular data. *Bot J Linn Soc*.,138: 123-144.

Dahlgren RMT, Clifford HT, Yeo PF (1985) The families of the monocotyledons. Structure, evolution, and taxonomy. Berlin: Springer-Verlag pp 92-128 .

Dhore MA, Joshi PA (1988) Flora of Melghat Tiger Rreserve. Technical series no.1 Directorate, Project Tiger Melghat, Partwada, Dist. Amravati. Maharashtra. 173-174 .

Duthie JF (1960) Flora of the Upper Gangentic plain Vol III. Botanical Survey of India pp. 321- 326.

Harvey PJ, Boulter D (1983) Isolation and characterization of the storage protein of yam tubers (*Dioscorea rotundata*). *Phytochem*, 22: 1687-1693

Johnson BL, Hall O (1965) Analysis of phylogenetic affinities in the Triticinae by protein electrophoresis. *Amer J Bot*., 52 (5): 506-513.

Khan MA (1992) Seed-protein electrophoretic pattern in *Brachypodium* P. Beauv. Species. Ann Bot 70: 61-68Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature*, 227: 680-685.

Mignouna HD, Dansi A (2003) Yam (*Dioscorea* Ssp.) domestication by the Nago and Fon ethnic groups in Benin. *Genet Resour Crop Evol*., 50: 519-528 .

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

Mignouna HD, Dansi A, Zok S (2002) Morphological and isozymic diversity of the cultivated yams (*Dioscorea cayenensis / Dioscorea rotundata* complex) of Cameroon. *Genet Resour Crop Evol*., 49: 21-29.

Odeigah PGC, Oboh B, Aghalokpe IO (1999) The characterization of Nigerian varieties of pepper, *Capsicum annuum* and *Capsicum frutescens* by SDS-Polyacrylamide gel electrophoresis of seed proteins. *Genet Resour Crop Evol*., 46: 127-131.

Patel RI (1968) Forest flora of Melghat. International Book Distributors, Dehra Dun, India pp 317-320.

Prain D, Burkill IH (1936) An account of the genus *Dioscorea* in the East, Part 1: The species which twine to the left. Ann Royal Bot Gard, Calcutta 14: 1-210.

Rohlf FJ (1987) NTSYS-pc Micro-computer programme for numerical taxonomy and multivariate analysis. *Amer Statistician*, 41: 330.

Smith BW (1937) Notes on the cytology & distribution of the Dioscoreaceae. *Bull Torrey Bot Club* 64 (4): 189-197.

Sokal RR, Micluner CB (1958) A statistical method for evaluating systematic relationships. Univ Kans Sci Bull 38: 1409-1438.

Wilkin P, Schols P, Chase MW, Chayamarit K, Furness CA, Huysmans S, Rakotonasolo F, Smets E, Thapyai C (2005) A plastid gene phylogeny of yam genus, *Dioscorea*: Roots, Fruits & Madagascar. *Syst Bot* 30 (4): 736-749.