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Distribution and Morphodiversity Analysis of Genus *Dioscorea* from India with special reference to Satpura Hilly Ranges and Western Ghat of Maharashtra State, India.

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Abstract

The genus Dioscorea is commonly known as yam, and the family Dioscoreaceae popularly called the yam family. Seven species, two variety of Dioscorea were investigated for establishment of phylogenetic relationships based on a morphological analysis by using NTSYSPC. Morphological dendrogram reveals two clusters in which species under section Enantiophyllum distributed distantly in the dendrogram. However, section Botryosicyos and Lasiophyton exhibit morphological affinity. The section Opsophyton maintains separate entity and was found to be outgrouped.

Key words: *Dioscorea, Distribution, Morphology, Phylogenetic analysis.*

Introduction

The state of Maharashtra has 307,713 km^2 total geographic area, of which 50,632 km^2 (16.45%) is covered by forest (FSI, 2013). In order to conserve biodiversified flora and fauna the government of India has established 29 tiger reserves covering an area of 58,620 km^2; 508 wild life sanctuaries covering 1,18,400.76 km^2 area and 97 national parks extended over 38,223.89 km^2 area. Melghat forest (Satpura Hilly Ranges) which was declared as a Tiger Reserve in 1974, is one of the best tiger reserves in Maharashtra. The *Flora of Melghat Tiger Reserve* reports 648 flowering plant species, belonging to 398 genera representing as many as 97 families (Dhore and Joshi, 1988). Later Bhogaonkar and Deverkar (1999) recorded 67 additional species of Angiosperms belonging to 10 genera.

The Western Ghats of Maharashtra spread from Saputara range in North, and continues past Goa to Karnataka. It is also known as the Sahyadri Range in the states of Maharashtra, Karnataka and the Malabar region. The area under Western Ghats includes area of 159,000 sq. km. Western Ghats includes Radhangari (Panhala) and Dajipur Bison sanctuary is situated on the border of Kolhapur and Sindhudurg districts, situated at an altitude of 977.2 m. The hill chain of the Western Ghats of India (8°–21°N, 73°–77°E) runs parallel to the western coast of India for over 1600 km. Vegetation cover in this area, mostly moist evergreen forest (Pascal, 1988). Kaas plateau, which is also known as a *"Kaas Pathar"* is also part of this region. It is situated in Sahyadri, 25 KM away from Satara district. Kaas is also known as a *Maharashtra`s valley of flowers.*

The family Dioscoreaceae is a natural group of tuber forming, tropical vines. The family is usually allied with the Liliales and placed near Amaryllidaceae. 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. The recognition of Dioscoreaceae as a family was due to R. Brown in 1819, which associated together the genera *Dioscorea*, *Rajania* and *Tammus* using Dioscoreae. The plants of the family Dioscoreaceae probably arose in the cretaceous period. The present days Dioscoreaceae are evolved from para-Dioscoreaceae in the presumable course of evolution. The rhizomatous Dioscoreas at one time had traveled through the intervening continents of Africa, and were unable to maintain a place about the Southern Atlantic from the close of the cretaceous period. The conversion of proto-rhizomatous condition to the present day tuberous Dioscoreas proceeds through *Testudinaria-Tamus* direction in South America (Prain and Burkill, 1936; Burkill, 1960).

The genus *Dioscorea* is commonly known as yam, and the family Dioscoreaceae popularly called the yam family. The family Dioscoreaceae is represented by 39 species in *Flora of British India* (Hooker, 1894). Under the family Dioscoreaceae in the forests of Satpura Hilly Ranges and Western Ghats, the section Combilium includes D. *aculeata*, section Lasiophyton with *D. hispida* and *D. tomentosa*, Opsophyton with *D. bulbifera* and *D. bulbifera var. bulbifera* , *D. pentaphylla* resurrection under Botryosicyos latter on and the last section Enantiophyllum represented by *D. oppositifolia*, *D. belopylla, D.alata, D.alata* var. *visoflora* and *D.walichi* (Duthie, 1960; Dhore and Joshi, 1988). These sections were introduced earlier by Prain and Burkill (1936) who were the basic contributors on the taxonomy of Dioscoreaceae.

The plant systematics and evolutionary study provide valuable knowledge of the origin and pattern of life. Genetic diversity is the diversity of the sets of genes carried by different organisms; it not only occurs between organisms of same population but also in organisms of different populations of the same species, those in different families, orders, kingdoms and domains (Heywood and Watson, 1995). Marked diversity was observed among the nine species of the genus *Dioscorea* in forests of Maharashtra and also within individuals of the same species except *D. hispida*. 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 (Dahlgren *et al*., 1985; Brunnschweiler, 2004). The morphological characters reflect the differences at the genetic level, and provide a base for inference regarding genomic relationships (Johnson and Hall, 1965). The systematist analyses phenotypes and their variation and often used this evidence for phylogenetic inference. Such inferences lead to state that "phenotypes have a specifiable relationship to unobserved genotypes" (Gottlieb, 1977). The morphological diversity in the Dioscoreales has led to confusion over its constituent families and generas (Dhalgren *et al*., 1985). Therefore, morphological diversity study was found most significant for the establishment of phyletic relationships in most of the taxons in general and in the nine species of *Dioscorea* occurring in forests of Maharashtra in particular.

Materials and Method

Study area

The present study was carried out in Satpura Hilly Ranges and Western Ghats of Maharashtra State for collection and morphological characterization of genus *Dioscorea* in particular and Jabalpur (Madhya Pradesh) and Bhubaneswar (Orissa) in general.

Collection of Plant Material

The genus *Dioscorea* is widely distributed in Satpura Hilly Ranges, Maharashtra, India (Latitudes 21^{o} 15' N and 21^{o} 45' N, Longitudes 76^{o} 57' E and 77^{o} 33'E and altitude 312 M to 1178 M above mean sea level) and represents five species namely *D.bulbifera*, *D. oppositifolia*, *D.hispida*, *D.pentaphylla*, and *D. belophylla*. However, species like *D. bulbifera*, *D. oppositifolia, D. hispida*, *D. pentaphylla*, *D. tomentosa*, *D. alata* and *D. belophylla* collected from hill chain of the Western Ghats of Maharashtra (8°–21°N, 73°–77°E) from the prominent vegetative place like Amboli, Ambaghat, Ajara, Radhanagari Dam, Kagal, Anuskura, Sawantwadi, Adur, Batkanangale, Gadhinglaj, Tillari and Panhalla in Kolhapur district lies in the Sahayadri Hills of Western India. While some species like *D. bulbifera* and *D. hispida* was collected from Kaas plateau, which is also known as a "Kaas Pathar" 25 KM away from Satara. However, *D. bulbifera*, *D. oppositifolia, D. hispida*, *D. pentaphylla* collected from Sinhgad and Mulshi, Lonawala, Khopoli near Pune; Mabaleshwar, Pachagani of Satara District and from Lonere, of Raigarh District. Moreover, some species like *D. bulbifera var. bulbifera* from Chandrapur; *D.alata*, *D.alata* var. *visoflora*, *D.hispida* from Jabalpur (M.P.) and *D.wallichi* from Bhuvaneshwar (K). The survey and collection was made during June to October every year. Fresh and mature plant parts including flowers, fruits, bulbils and tubers were collected from the field. Specimens were made into herbarium sheets following Jain and Rao (1977) and identified using literature available and from Botanical Survey of India, Pune.

Morphological phylogenetic analysis

The phylogenetic analysis was carried out by constructing unitary data matrix as on the basis of morphological characters as per the method of Nei and Li (1979). The unitary data was analyzed for each marker by using NTSYS-pc version 2.0 (Rohlf, 1998) to generate similarity coefficient. The matrix was subjected to Unweighted Pair Group Method for Arithmetic average analysis (UPGMA) (Sneath and Sokal, 1973) to generate dendrogram using average linkage procedure.

Observations and Results

Distribution

The *D.bulbifera* and *D. bulbifera* ver. *bulbifera* is widely spread in Satpura Hilly Ranges and Western Ghats of Maharashtra and reason for this wide distribution these plant produces more bulbils this is a vegetatively propagated organ, usually 50 to 80 bulbils on full grown plant and have bitter taste, because of this the tubers are not eaten by wild animals hence there is no disturbance by wild animals. Although *D. oppositifolia* and *D. alata* is having bulbils like that of *D.bulbifera* but it supersedes all other species with high rate of seed setting. The tubers of the *D. oppositifolia*, *D. alata*, *D. alata* ver. *vilosiflora* species are very tasty and eaten by wild animals therefore, frequency of these species was found to be lowest with respective to other species in the forests of Maharashtra. Likewise tubers of *D.pentaphylla* and *D. alata* are tasty and eaten by wild animals. The seed-setting rate in this species is moderate. Flowering and seed setting in *D.hispida* is lower than *D. oppositifolia* , *D. alata* and *D.pentaphylla* but the frequency of *D.hispida* is more than above two species because of multiple tubers (4-5) produced by a single plant , another most important character of this species is the bitter taste of tubers like that of *D.bulbifera;* this minimizes the disturbance by wild animals. Seed setting in *D. belophylla* is the lowest of all four species but the plant frequency is more than *D. oppositifolia*, *D. alata* and *D.pentaphylla* because of the fact that each tuber of *D. belophylla* sprouts all around its length hence number of plants produced by a single tuber is the largest among all the species under observation. However, *D. alata*, *D. walichi* and *D. tometosa* exhibited less density because of its highly medicinal properties of tubers and these species were having high agro-economic value (Figure-1).

Morphological variations

Morphologically the tubers of *D.pentaphylla* ,*D. oppositifolia*, *D. alata*, *D. alata* ver. *vilosiflora* and *D. belophylla* resemble in characters such as long, slender form, inside fleshy and full of mucilage with white fibres. It is to mention that *D. oppositifolia*, *D.*

alata, and *D.pentaphylla* these plants have food value and their tubers are eaten by wild bears and tribes of the area, but tubers of *D. belophylla* are smaller in size than those of *D. oppositifolia*, *D. alata* and *D.pentaphylla*.

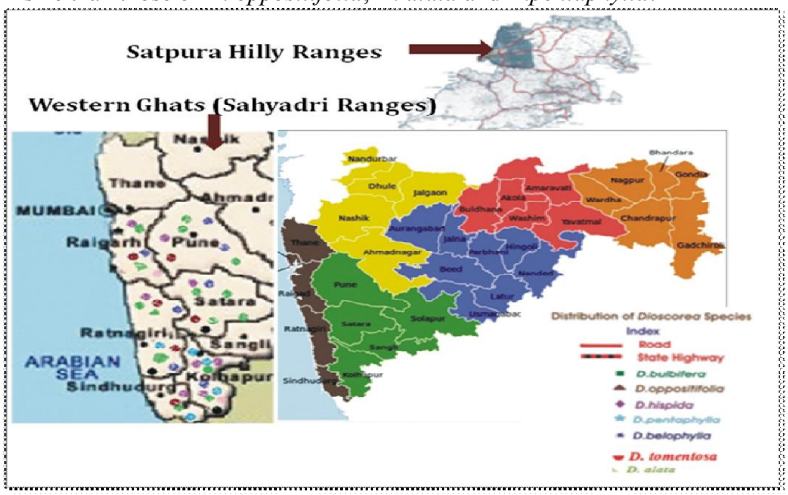

Fig 1: Distribution of genus *Dioscorea* in forests of Satpura Hilly Ranges and Western Ghats

The typical leaf of *Dioscorea* is simple with reticulate multicostate convergent and the simple leaf converts in to a compound leaf. The leaf of *D.bulbifera*, *D. bulbifera* ver. *bulbifera*, *D. oppositifolia D. alata*, *D. walichi* and *D. belophylla* are simple. The shape of leaf in these species is cordate with reticulate multicostate convergent venation, and lathery however, cartilaginous in *D. oppositifolia* and *D. walichi*. Lathery with mucilage and polyphenols in *D. belophylla*, where as in *D.pentaphylla* and *D.hispida* leaf is multifoliate. *D.hispida* having small white hairs on leaf is unique of its kind as no such character visible in other species of *Dioscorea*. Although the leaf of *D.pentaphylla* and *D. tomentosa* is multifoliate like *D.hispida* however in former species venation is reticulate unicostate and penninerved, while in latter it is reticulate multicostate convergent. During the course of evolution there may be a gradual change in form of leaf from simple as in *D.bulbifera*, *D. bulbifera* ver. *bulbifera*, *D. oppositifolia D. alata*, *D. walichi* and *D. belophylla* with reticulate multicostate convergent venation to multifoliate with the reticulate multicostate convergent as in *D.hispida*. Besides, there is a exhibition of highest degree of ramification and gradual change of trifoliate condition to multifoliate and multicostate convergent to unicostate penninurved as in *D.pentaphylla*.

Phylogenetic Analysis

Dendrogram reveals that *D.belophylla* from Melghat with little variations in vein color and from Western Ghats clustered together. However, *D. pentaphylla* and *D. tomentosa* of section Botryosicyos and Lsiophyton respectively showed close resemblance. *D.wallichii* from Jabalpur (M.P.) and Bhubaneshwar (A.P.) on the basis of morphology exhibited close affinity. Moreover, *D. hispida* from Melghat and from Jabalpur are appears to be genetically closer but separated from *D. pentaphylla* and *D. tomentosa*. The *D. alata* and *D. alata* var. *visoflora* was cluster together in sub-cluster of cluster-I. However, sub-cluster-III of cluster-II brings *D. oppositifolia*, *D. bulbifera* and *D. bulbifera* var. *bulbifera* from Melghat and from Western Ghats of Maharashtra State clustered together.

On the basis of morphology simple leaf species were found to be sandwiched in between compound leaf species that is penetration of *D. wallichii* in between trifoliate and pentafoliate species such as *D. tomentosa* and *D. pentaphylla* both have single nerve respectively and *D. hispida* trifoliate with three mid ribs. The dendrogram exhibited the braking of the section Lasiophyton and Enantiophyllum. The Lasiophyton species *D. tomentosa* separated morphologically from *D. hispida*. However, Enantiophyllum species *D. wallichii* separated from *D. alata*, *D. alata* var. *visoflora* and *D. oppositifolia* (Figure-2).

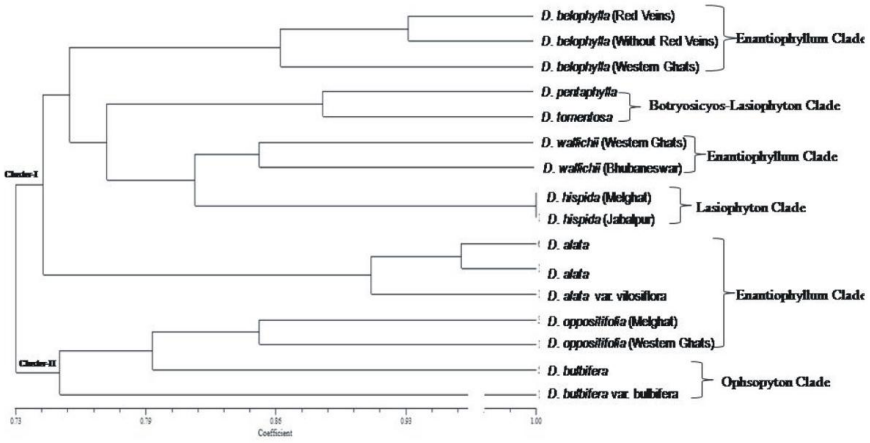

Fig 2: Phylogenetic analysis *Dioscorea* species based on morphology.

Discussion

Distribution

The Dioscoreales comprises a tropical group of families scattered over all the chief continents with a possible centre in Southern Asia and Indonesia. The plants of the order are terrestrial and range from shade herbs on the forest floor to climbers in scrubby or rocky habitats. Climbers sometimes are strongly adapted to arid conditions e.g. *Dioscorea* sect. *Testudinaria* (Dahlgren *et.al.*, 1985). This great tropical genus *Dioscorea*, distributed through the warmer parts of the world, the wide species are very few and in fact there is only one species i.e. *D. bulbifera* which claim even to approach the range of the whole genus (Good, 1953). Man has been cultivating a variety with very large bulbils such as *D. bulbifera* var. *sativa*- which was brought to India from Far East in the middle of the last century and latter to Europe. This species in particular gains by means of its bulbils a quick way of securing establishment (Burkill, 1960). During survey of Satpura Hilly Ranges and Western Ghats although nine species were found to be distributed in different parts of the forest, the frequency of *D. bulbifera* is the highest with respect to other eight species.

All over the Satpura Hilly Ranges and Western Ghats forest there are dense crowded patches of vegetation, broken by the pokets of less dense plant species; this situation is conducive for the growth of *Dioscorea* species, as the climbers get sufficient light for their growth was noticed by present investigator. *Dioscorea* thrives well, where the forest is broken and a few hold their own even in the heart of the most densely afforested countries (Burkill, 1939). Species of *Dioscorea* are widespread in the tropics and subtropics and are most frequently encountered as climbers which perennate by rhizomes or tubers in the forest margins and more open habitats (Wilkin, 2001).

The section Enantiophyllum represents by species like *D. oppositifolia*, *D. belopylla, D.alata, D.alata* var. *visoflora* and *D.walichi* is restricted in to some pockets in the forests of Satpura Hilly Ranges and Western Ghats. Reason for the reduce frequency of this species is due to tasty tubers eaten by tribal people and by wild animals. Where there is a sufficient light and the plants are fully or partially exposed, there is healthy growth of the plants, however low light penetration at dense vegetation or in case the young plants are covered by bushy shrubs like *Lantana camera*, the frequency of these species was found to be less.

Distribution of *D. belophylla* is more to those of *D. oppositifolia*, *D.alata, D.alata* var. *visoflora* and *D.walichi*, due to multiple shoot formation. *D. hispida* of section Lasiophyton is the second largest species in distribution and is due to multiple non edible tubers formed by a single plant that leads to increase in number of plants in a particular habitat and is restricted to deep valleys of Satpura Hilly Ranges and Western Ghats with relatively high humidity. Unfavorable climatic condition may be the reason for disappearance of *D. hispida* from Ceylon and the Andaman islands, thus indicating that cultivation by man extended *D. hispida* further towards the subtropical forest of East Africa than its own power of spreading (Burkill, 1960). The tubers of *D. hispida* are poisonous, and the juice of tubers, when mixed with antiaria poison, is used as arrow poison (Dahlgren *et al.*, 1985).

Morphological variations

The main systematic challenge in terms of biodiversity in Dioscoreales is *Dioscorea* and is also by far the most geographically widespread taxon. Being almost ubiquitous in tropical and sub-tropical regions with a few species found in temperate areas (Wilkin *et al*., 2005). The problem on phyletic relationship was established by Burkill (1960) on the basis of nodal anatomy, geographical distribution, areal and underground parts and morphodiversity; the problem in this regard was unsolved till 1985. Solution to problem to a larger extent was given by Dahlgren *et al*. (1985) considering structure and taxonomy in Dioscoreales; the order Dioscoreales included seven families: Dioscoreaceae, Petermanniaceae, Smilacaceae, Stemonaceae, Taccaceae, and Trilliaceae. The considerable morphological diversity within Dioscoreales led to confusion over its constituent families and genera. The genus *Dioscorea* presented a challenge to systematics for many years due to its great morphological diversity, dioecy, and small flowers (Wilkin *et al*., 2005).

The two species, *D. rotundata* and *D. cyenensis* cultivars were not grouped according to their species designation on the basis of RAPD patterns (Asemota *et al.*, 1996). But later on the yellow (*D. cyenensis*) and white (*D. rotundata*) form of Guinea yam were divided into two clearly defined groups according to their species designation (Ramser *et al*., 1997). Likewise, morphological dendrogram reveals two clusters in which species under section Enantiophyllum distributed distantly in the dendrogram. However, section Botryosicyos and Lasiophyton exhibit morphological affinity. When compound leaf species, *D. tomentosa* and *D. pentaphylla* of the section Lasiophyton are cluster together, *D. hispida* in spite of belonging to same section was placed in another cluster, showing its genetic dissimilarity with above two species (Asha *et al*., 2006). The species under Botryosicyos-Lasiophyton clade such as *D. pentaphylla* (Earlier under Lasiophyton and now resurrection under Botryosicyos) and *D. tomentosa* have pentapholiate, unicostate reticulate vein leaves and tripholiate, multicostated, convergent and peninerved leaves respectively. The section Opsophyton maintains separate entity and was found to be outgrouped.

Conclusion

In conclusion the species belonging to the different sections such as section Lasiophyton, Enantiophyllum, Opsophyton and Botryosicyos were found to be grouped according to their species designation on morphology based cluster analysis but impregnation of the section Lasiophyton with Enantiophyllum is a major dilemma. The character based matrix analysis was found to be significant and resolve the dilemma of impregnation of these sections needs to extend further by adopting combine cluster analysis on molecular level.

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