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**RESEARCH ARTICLE** 

# CELLULAR ARCHITECTURE IN SELECTED SPECIES OF GENUS *DIOSCOREA*, KERALA, INDIA.

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## Manuscript Info Abstract

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The cellular architecture is a clue to the species and variety differences in this group. A special, spiral, blend ending, corner located 'Dioscoreal raphides' present in the pith cells of D. pentaphylla (5-3leaves) and D. bulbifera (Red stem). Needle shaped bundles of raphides present in pith cells of D. bulbifera (Green stem) and D. kalkapershadii. Large amount of chloroplast present in pith cells of D. tomentosa, which also bearing sclerids around the phloem tissue and in D. kalkapershadii, sclerids are present around the vascular bundle. Large and small starch grains along with different amounts of chloroplast are common in this group. A ring of cells in the inner most hypodermis contains a mass of starch grains present in D. bulbifera (Red stem) and D. kalkapershadii. Large phloem cavities formed after disintegration of phloem cells. Evolutionary changes from Eustele to Atactostele of vascular bundles have seen in this group of plants. Vascular bundles are monocot mesarch and broken ring arrangement in D. kalkapershadii, D. pentaphylla and D. tomentosa. Unpaired metaxylem present in D. bulbifera and distantly paired metaxylem in D. hispida.

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### Introduction

A species without enough genetic diversity is thought to be unable to cope with changing environments or evolving competitors and parasites (Schaal et al., 1991) and also the ability of a population to respond adaptively to environmental changes depends on the level of genetic variability or diversity it contains (Ayala and Kiger, 1984). 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 of that species.

Yams (*Dioscorea* species) are perennial trailing rhizome plants, which belong to Dioscoreaceae family. The genus *Dioscorea* includes over 600 species (Ayensu, 1972), 50 species are reported from India, and 17 species are reported from Kerala. A number of *Dioscorea* wild species are the source of compounds used in the synthesis of sex hormones and corticosteroids and cultivated species are the source of food in some tropical countries.

*D.bulbifera* L. is the true yam family in Dioscoreaceae and it is known as 'Varahi' in Sanskrit, 'Kachil' in Malayalam and it is very common in the name 'Air potato'. *D.pentaphylla* L. is another species in the yam family known by a 'Nootakizhangu' or '5 leafed yams', and they posses' compound leaf with 3 or 5 leaflet. *D. tomentosa* J. Konig ex Spreng. and *D.hispida* L. are the two species which posses compound leaf with 3 leaflets and the *D.hispida* is commonly known as 'Asiatic bitter yam', a poisonous tuber, but a savior during famine period. It is the only species under the genus *Dioscorea* which posses trinerved leaflets. The *D. kalkapershadii* L. is commonly known as 'Nara, or mullenkaavathu' posses' compound leaf with 5 leaflets.

Genus *Dioscorea* shows species diversity in India. Many species are similar in appearance but some were in complex. *D. pentaphylla* and *D. kalkapershadii* have numerous types of leaves, 3 - 5 in *D. pentaphylla* and 3-7 in *D. kalkapershadii*. The species differences are much less, at the same time *D. bulbifera* consist of two varieties-red and green stemmed species. Floral characters are more or less similar but vegetative leaf and stem morphology are different. More over Elsamma Joseph, *et al.*, (2013) had reported nine unidentified samples from the same area. So the cellular architecture of *Dioscorea* species has become a solution for the systematic problem in this group.

The classification of plants under variety level is not an easy one as in case of *Dioscorea* and the classification under variety level is not completed and accepted. The comprehensive studies related between the species of *Dioscorea* have not been carried out till date. Based on all these axioms the present study focus the 5 selected species of *Dioscorea* (*D. bulbifera*, *D. pentaphylla*, *D. hispida*, *D.kalkapershadii*, and *D. tomentosa*) found in different parts of Thrissur district, Kerala, was carried out. The studies include cell architecture and anatomy of stem.

#### Materials and methods

Stem of *D.bulbifera* (Green stem), *D.bulbifera* (Red stem), *D. pentaphylla* (3-leaflets), *D. pentaphylla* (5- leaflets), *D.hispida* (3-leaflets), *D. kalkapershadii* (3-7 leaflets) and *D. tomentosa* (3 leaflets) were studied. These are collected from five natural distribution zones of various regions of Thrissur district, Kerala. The collection period range from 2013 August to 2014 March, for experimental purpose. Then the plants were identified.

#### **Results and discussions**

*D. bulbifera* and *D. pentaphylla* are the three widely distributed genera among the family Dioscoreaceae .When look in to the sample the *D. bulbifera* and *D. pentaphylla* are more prominent than *D. tomentosa*, *D. hispida* and *D. kalkapershadii*.

All the Dioscorea species with round or hexagonal stem. Most of the species of this genus have spines on their stem. If spines absent, they produce terete nature on their stem. The prickling stems are D.pentaphylla, D. hispida, D. tomentosa and D. kalkapershadii where as D. bulbifera produce terete stem. The T.S of the stem shows epidermis cell with thick wall and barrel shaped cells. This genus bears two celled hairs, one short basal cell and elongated pointed, conical upper cell. The inner most cells of hypodermis store large amount of starch grains in D.bulbifera (Red stem) and D. kalkapershadii. Inner to epidermis a hypodermal region which consists of six layers of round cells which bears chloroplast. Inner to hypodermis is a sclerenchymatously delimited cortex. The sclerenchyma cells have space gap in their walls. The cortex consists of vascular bundles and pith. The vascular bundles are arranged in two or three concentric layers. The vascular bundles are more or less similar to that of dicot stem, but they are typical monocot. The central large bundles are arranged in broken ring manner in both 5 leaf species and 3 leaf species. In 5 leaf species the bundles are "Bicolletral" and "mesarch". The paired small xylem vessels are present at the anterior and the posterior end of the metaxylem of large monocot vascular bundles. The small xylem vessels with multiple perforation plates and metaxylem vessel with simple perforation plates are present. The outer most rings also consist of small 5 vascular bundles in between large vascular bundles and they connected with the sclerenchymatous cortex cells. The stems of D. pentaphylla, D. hispida, D. tomentosa and D. kalkapershadii contain 5 large vascular bundles. The paired large vascular bundles are present in these species, but others differ from *D.pentaphylla* by means of small protoxylem only present in the anterior part of large vascular bundles. The connected peripheral layer is normal monocot type and represent in between the space of large vascular bundles (Table 1., Plate 1.).

The 3-7 leaf complex now morphologically and anatomically different from each other. The 5 leaflet complex is now identified as *D. kalkapershadii*. The 3-5 leaf bearing species identified as *D. pentaphylla* Van Rheed in 1686, from the Malabar area reported two species of *Dioscorea* in Hortus Malabaricus, Vol 7, 33-35. Rheed described 3 leaflet species as *D. triphylla* (Vol 7, 33) and five leaflet species as *D. pentaphylla* (Vol 7, 35). In our study the *D. kalkapershadii* only shows 3-7 leaves. The three leaved *D. pentaphylla* shows five leaves at their maturity. So the 5 leaflet *D. pentaphylla* and 3 leaflets *D. pentaphylla* are same. The 3-7 leaf producing *D. kalkapershadii* and only three leaves producing *D. pentaphylla* are morphologically and anatomically different. So the Linneas species *D. pentaphylla* is most suitable for 3 and 5 leaflet species of *D. pentaphylla*. Both three leaf producing *D. pentaphylla* and *D. kalkapershadii* are similar in appearance but different their leaf color, pale green in *D. pentaphylla* and dark

green in *D. kalkapershadii*. The three leaved *D. hispida* is morphologically and anatomically different from other three leaved *D.pentaphylla*, *D. kalkapershadii*, and *D. tomentosa*. The *D. pentaphylla* and *D. kalkapershadii* produce elongated tubers. But in *D. hispida* species produce globose tubers and male plants flowers in June itself. This species is very much morphologically similar to that of *D. dumatorum*, an African species of *Dioscorea*. But close observation and morphological peculiarities of this species is helped to identify as *D. hispida*. In stem anatomy of the *D. hispida*, the paired large vascular bundles not arranged in 'Y' or 'V'shape. The large vascular bundles are not clear and large as *D. pentaphylla* and *D. kalkapershadii*. All the three leaflets are tri-nerved.

The single or one species of D. bulbifera with green stem and red stem are more or less similar, their anatomical studies shows dissimilarities. In the anatomy of stem shows a typical monocot stem with scattered vascular bundles arrangement with one large, unpaired xylem vessels and two protoxylem points present towards the posterior side. Large phloem cavities formed after disintegration of phloem cells. It is look like a typical monocot stem. The corners of each cell consist of spirally arranged, blunt end, elongated raphides present in D. pentaphylla and D. bulbifera (Red stem). It is not known to the plant cells so it is called as "Dioscoreal raphides". D. kalkapershadii, D. pentaphylla, and D. tomentosa posse's dicot stem like large vascular bundles 5+5 arrangement. D. pentaphylla (3 leaves) contains 5 large vascular bundles. Large vascular bundles of D. kalkapershadii attached to the sclerenchyma endodermis cells. Large vascular bundles of D. pentaphylla and D. tomentosa free from the sclerenchyma endodermis cells. Large vascular bundles of D. tomentosa are broader and shorter than D. pentaphylla. D. pentaphylla, D. tomentosa and D. kalkapershadii shows phloem in both sides of large metaxylem pair. In these species anterior side of bundle a pair of phloem cells and posses one group of phloem present at the posterior end of large vascular bundle. The phloem cells bordered by sclerids in D. tomentosa and the entire bundles are covered with sclereids in D. kalkapershadii. Bundles of needle shaped raphides present in D. bulbifera (Green stem) and singly arranged needle raphides in D. kalkapershadii. Mucilaginous canal cells present in all species. The Chloroplast is common in hypodermal cells of all species. D. tomentosa bearing pith cells with large amount of chloroplast, but others bears sparsely in pith cells. Large starch grains are present in D. bulbifera (Green stem). Small starch grains are present in D. kalkapershadii and D. bulbifera (Red stem). Very small and rectangular or square raphides present in D. kalkapershadii. Both sides of protoxylem lacunae, phloem with lacunae are also present in compound leaf species (Plate 2.).

Characters > Species name	Vascular bundle	Paired m.xylem	phloem	Sclereids in V.B.	Starch grain and raphides	Mucilage canal	Chloro- plast in pith
D. kalkapershadii	Bicolleteral, mesarch, broken ring, limited, 5+5.	Paired lateraly.	Both ends.	Present around the vascular bundle.	Needle raphides,Small starch grains.	Present.	Absent.
D. pentaphylla (5 leaves)	Bicolleteral, mesarch, broken ring, limited,3+3+5.	Paired lateraly.	Both ends.	Absent.	Dioscoreal raphides,starch grains absent.	Present.	Absent.
D. pentaphylla (3 leaves)	Bicolleteral, mesarch, broken ring, limited,3+3+4.	Paired lateraly.	Both ends.	Absent.	Dioscoreal raphides and starch grains absent.	Present.	Absent.

Table I -Cellular p	eculiarities and	anatomical	characters	in	Dioscorea	species
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D. hispida	Broken ring, limited.	Distantly Paired lateraly.	one end.	Absent.	Abesnt.	Present.	Little.
D. tomentosa	Bicolleteral, mesarch, broken ring, limited, 5+5.	Paired lateraly.	Both ends.	Present around phloem.	Absent.	Present also in xylem.	Largely present.
D. bulbifera ( Red stem)	Scattered, linear,numerous.	Unpaired, back to back.	No such arrageme nt.	Absent.	Dioscoreal raphides, small starch grains.	Present.	Absent.
D. bulbifera (Green stem)	Scattered, linear,numerous.	Unpaired, back to back.	No such arrageme nt.	Absent.	Needle like raphides and large starch grain.	Present.	Present.

#### **Evolutionary significance**

Looking through the five species anatomical peculiarities the vascular bundles are much more similar to that of dicot stem with increasing number of leaves. *D. kalkapershadii* and *D. tomentosa* shows greater affinity towards dicot plant, with palmately compound leaves. Each leaflet bears only one midvein and broken ring arrangement of vascular bundles. The large vascular bundle shows 'Bicolletral' appearance. So that *D. kalkapershadii* and *D. tomentosa* are the most primitive among the five species, which are also very rare occurrence in their habitat. *D. pentaphylla* stands next to first two species, but shows greater affinity towards *D. kalkapershadii* in vascular bundle, 'Bicolletral' nature and compound leaves. *D.hispida* shows small similarities to *D. bulbifera* and greater to *D. pentaphylla*. The leaflets are trinerved, vascular bundle are shows small distinction with large and small vascular bundle layers. The presence of distantly paired nature of vascular bundles present in other two species with compound layers is absent or not regular in *D.hispida*.

*D. bulbifera* shows typical scattered vascular bundle. Both varieties of *D. bulbifera* are different in their vasculature and storage cells. The species of *D. bulbifera* and *D. pentaphylla* are very common and showing high degree of mucilaginous content in their stem. Mucilage content is greater in *D. bulbifera* than *D. pentaphylla*.

The similarities of *D. hispida* with *D. dumatorum* an African species is very significant. The *D. bulbifera* and *D. pentaphylla* shows wide distribution pattern in world. So the evolutionary significance also indicates that *D. kalkapershadii* will disappears in the near future. In The Flora of Presidency of Madras comment this species as a form of *D. pentaphylla*. So the need of conservation of this species is very important.



Plate 1:

Stem T.S of six selected *Dioscorea* species. (A) *D. bulbifera*- Red stem, (B)*D. bulbifera*-Green stem, (C) *D. kalkapershadii*, (D) *D. tomentosa*, (E) *D. hispida*, (F) *D. pentaphylla* (1-Epidermis, 2- Cortex, 3- Outer ring of vascular bundles, 4- Metaxylem, 5- Phloem)



Plate 2

(A) Epidermis, (B) Mucilage canal, (C) Chloroplast in pith cells, (D) Sclereids, (E) Aleurone grain, (F) Needle raphides, (G) Raphides present in the edges of cell wall, (H) Dioscoreal raphides

#### Key to studied species with respect to the cellular architecture.

- 1. Leaf simple and stem with unpaired metaxylem and scattered v.b. ......2
- 1. Leaf compound and stem with paired metaxylem and broken ring v.b. ......3
- 2. Innermost metaxylem with simples perforation pith with large mucilage canal and no Dioscoreal raphides
  - ...... D. bulbifera (Green stem)

2. Innermost metaxylem with simple perforation and Dioscoreal raphides

*D. bulbifera* (Red stem)

5. Pith with Dioscoreal raphides and starch grains absent ......D. pentaphylla

5. Pith with very Needle shaped raphides and small stach grains

.....D. kalkapershadii.

#### Conclusion

By this study can draw the evolution line, the primitive *D. kalkapershadii* to the latest *D. bulbifera*. Evolutionary change from Eustele to Atactostele of vascular bundles has seen in this group of plants.

D. kalkapershadii  $\rightarrow$  D. tomentosa  $\rightarrow$  D. pentaphylla  $\rightarrow$  D. hispida  $\rightarrow$  D. bulbifera.

In *D. bulbifera* (Red stem) and *D. pentaphylla* the tangential wall corners of each pith cell consist of spirally arranged, blunt end, elongated raphides. They look like the double helix DNA, but the both ends are free. It is not known to the plant cells. When searched for occurrence of this type of raphides among the angiosperms, there were no record of the raphides as seen in *D.bulbifera* (Red stem) and *D. pentaphylla*. So, it is a new record of raphides. For the unique raphides of *D. bulbifera* (Red stem) and *D. pentaphylla*, a new term "Dioscoreal raphides" has been proposed in this study.

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