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Morpho-histological studies in early developmental Leaf and Cotyledon of *Adenium obesum* (Forssk.) Roem. and Schult

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ABSTRACT

The present study reports the morphological and anatomical study of leaf and cotyledons of seedling of *Adenium obesum* L. It is one of the most important cultivated garden plant of Apocynaceae in India. It is also known as desert rose as it is a xerophytes plant. *Adenium obesum* is a succulent flowering plant in the dogbane family, Apocynaceae. It is an evergreen or drought deciduous succulent shrub (which can also lose its leaves during cold spells and can grow to 1–3 m (3.3–9.8 ft.) in height. It has pachycaul stems and a stout, swollen basal caudex (obese stem), because of which its look like a bonsai and so economically important horticulture plant. In the present study seeds were collected and shown in the pot. Seedlings were collected with cotyledons at 15th day for morphological and anatomical observation. Morphological characters for cotyledon and leaf such as length, width, apex, angle showed that these characters are correlated with both leaf and cotyledon each other. In the anatomical observation epidermis layers and mucilage layers, palisade tissues, spongy tissues, calcium oxalate crystals are observed. The vascular bundles of the both organs are bicollateral.

Keywords: *Adenium obesum*, Apocynaceae, morphological, anatomical

I. INTRODUCTION

A juvenile stage of the plant after the germination is known as seedling. Jackson (1928) defined seedling as a young plant produced from seed, different to the artificially propagated plant. A widely accepted definition of seedling was given by de Vogel (1980). He defined 1he seedling as a very juvenile stage of the plant after the germination. The term seedling is normally used for the woody plants. From the beginning of germination up to a stage where it is 25 – 50 cm high. When larger, it is called a young plant. Until it is over about one-meter-high and then it is called sapling (de Vogel, 1980). According to Troup (1921) the seedling is the most critical stage in the life of a tree.

Several changes occur in seedlings during the early age. During the early phase of development, the endosperm of seed or food storing cotyledons provide nutrients and thus support till the development of shoot system. When the cotyledons are shed, their functions are taken up by the newly developed leaves. The leaves of a seedling may differ from the adult plant. (Jackobs, 1966; Guillaumin, 1910). For example, the first produced leaves may be simple in seedlings instead of compound or the size is small, type of venation is different and for certain species the lobes are not

prominent as in the adult stage. Because of such morphological differences in the juvenile plant from adult it is difficult to recognize the plants when they are in the seedling stage. So that anatomical study is must to understand the internal differences between leaf and cotyledon at juvenile stage of plants.

In the second half of the 19th century the study on the anatomy of the seedling was started (de Vogal, 1980). The main early workers in the field of the seedling anatomy were Sargeant (1902), Gatin (1906), Hill & de Frain (1910), Thomas (1914), Artier (1920) and Compton (1912). Later, many workers studied the seedling anatomy and some of them had also contributed on the vasculature of seedlings. The morphology and anatomy of the seedlings of large number of genera of the families of dicotyledon have been studied. However, our knowledge about the seedlings of *Adenium obesum* of Apocynaceae is very incomplete and the study of it is still in the exploring stage. Characters of the seedlings offer more valuable clues." Identification of seedlings is of great importance to plant breeders, ecologists, agronomists, foresters and others. The study of the morphology of seedlings often provides characters of taxonomic value (Lubbock,1892).

The present paper includes the study of external and internal structure of lamina, midrib and petiole of cotyledon and first form leaf of seedling of *Adenium obesum*. The *Adenium* is an incredibly fun genus to work with and is really one of the iconic caudiciforms in the trade. As this plant is look like as bonsai because of its short, slow growing and obese stem, it is very interesting garden plant for researcher. There are very scanty of knowledge about Morpho – histology of its leaf and cotyledon, require more focus in it

II. METHODS AND MATERIAL

The seeds of Adenium obesum family Apocynaceae were collected from Hari Nursery, Surat. Healthy seeds were selected and sown in separate plots. Sufficient water was sprinkled regularly to facilitate germination. The seedlings at different developmental stages were collected from the garden and brought into laboratory. They were thoroughly washed with tap water, cleaned. The Leaves & Cotyledon were cut and fixed in Formalin - Acetic acid - Alcohol (FAA) (Jensen, 1982) and aspirated to remove air. Free hand cut sections for the investigation. They were stained with Safranin. They were directly mounted with glycerine jelly. Photographs were taken on Carl Zeiss photomicroscope with planophotochromatic objectives. Kodak 100 ASA - 35mm color negative film and yellow, daylight or yellow filters were used. The photographs of different parts of seedling with cotyledon and first leaf were taken by Nikon FM2 camera using same color negative film.

III. RESULTS AND DISCUSSION

1. Morphology

The morphological observations are outlined and presented in Table: 1 & Fig. 1

Table: 1 morphological observation of seedlings

Taproot	short, 1 to 2 cm, sturdy, flexuous, creamy brown, with rather few, quite short, shortly branched, creamy brown side roots
Hypocotyl	hardly elonging, slow growing, terete, swollen from middle and narrow from ends, barrel — shaped, 3 to 4 cm long, pale green, glabrous, highly fleshy.
Epicotyl	very short, slow growing, soft, fleshy, narrow towards top, terete, to 0.5 cm. long, green, glabrous.
Cotyledons	2, opposite, somewhat succulent in the 5th-6th leaf stage dropped, exstipulate, almost sessile, fleshy, dark green, glabrous.
First pair of leaves	First two leaves opposite, simple, exstipulate, almost sessile, sub
Next leaves	spirally arranged, simple, exstipulate, almost sessile, subcoraceous, green, glabrous

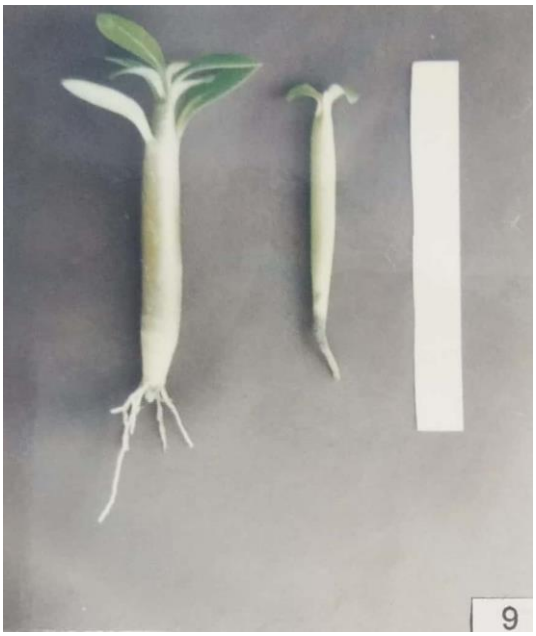

Fig. 1 Seedlings of *Adenium obesum.*

2. Anatomy

2.1 Anatomy of Leaf Lamina, Epidermis

In the leaf of *Adenium obesum* adaxial epidermal cells are larger than the abaxial ones. The adaxial and abaxial epidermis are one layered, consisting of rectangular, squarish, tubular or barrel shape thin walled cells (Fig. 1, Table 2). The cell wall of epidermal cells may be sinuous or non -sinuous on both the surfaces. The outer walls of epidermal cells are cutinized. The cuticle is thick and slightly wavy

Table:2 Size of the cells and type of cell wall in leaf and cotyledonary epidermisin *Adenium obesum*

Plant	Size of epidermal cells (µm)				Cell wall	
	Adaxial Surface		Abaxial Surface		Adaxial Surface	Abaxial Surface
	L	B	L	B		
Leaf	74.0	56.4	65.6	41.2	Non sinuous	Non sinuous
cotyledon	71.6	51.2	64.0	38.8	Non sinuous	Sinuous

2.2 Anatomy of Leaf Mesophyll

In most of the investigated species it is well differentiated into palisade and spongy tissues. In *Adenium obesum* also 2 - 3 layers of short palisade cell layers are present. The length of palisade cells is highest in the outermost layer which subsequently may decrease in the layers towards the inner side.

The abaxial surface in most of the investigated species consists of spongy tissue. The spongy tissue consists of irregular or circular cells elongated in the direction parallel to the leaf surface. They have thin cell walls, larger intracellular spaces and contain large number of chloroplasts.

2.3. Anatomy of Leaf Vascular system

Since the leaves are pinnately veined, they are provided with a thickest median or 1° vein to which 2° veins are laterally connected. They are connected by still smaller veins (30) to which other smaller veins (4°) are connected. The 10 vein can be observed in transactions as a central vascular bundle of midrib and the remaining different orders of veins or small bundles in lamina, which are embedded in the mesophyll. Normally these bundles are transverse, oblique or longitudinal and contain xylem and phloem. The smallest bundle (4 and ultimate branches) contain only xylem, mainly composed of tracheids. These bundles are devoid of the supporting tissue of well-organized bundle sheath.

2.4 Anatomy of Leaf Midrib

Midrib epidermis resemble to that of lamina the outer surface of epidermis is cutinized. Cuticle is thick, slightly wavy. (Figs. Midrib, ground tissue in *Adenium obesum* is differentiated in to adaxial and abaxial hypodermis of collenchyma and cortex of parenchyma stellate crystals of calcium oxalate are observed in the parenchyma of *Adenium obesum* (Fig. 2(601).

Midrib vasculature consists of a single larger strand located in the central part (Figs.2(601), 3(592), 4-6(603- 605).

It is median vein (1). It is crescentic in many plants but C - shape in *Adenium obesum* Figs, 3(592), 4-6(605- 606). It is conjoint collateral or bicollateral and open with outer phloem facing towards abaxial and inner phloem towards adaxial surface and is devoid of well-organized sheath.

Cambium cells present between xylem and outer phloem remains active up to limited period. They form more cells of xylem to adaxial and scanty phloem to abaxial side (Figs.3 (592).

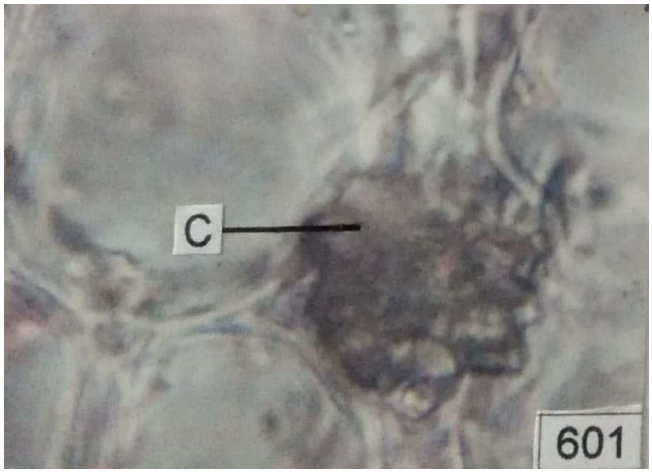

Fig.2 (601): Transections of midrib of leaf. X570. (C- crystals)

2.5 Anatomy of Leaf Petiole

The leaves are petiolate in many Apocynaceae plants. There is very short petiole observed in *Adenium obesum*. Epidermis is unilayered, made up of rectangular or tubular cells; smoothly cuticularised.

The ground tissue is paranchymatous. The vascular systems consist of a single central vascular strand. It is C -shape and cambial activity is similar to the midrib

2.6 Anatomy of Cotyledon Lamina, Epidermis

In transactional view, the cotyledons of all the species studied are elliptic in out line. The cotyledonary margin may be sub obtuse or rounded.

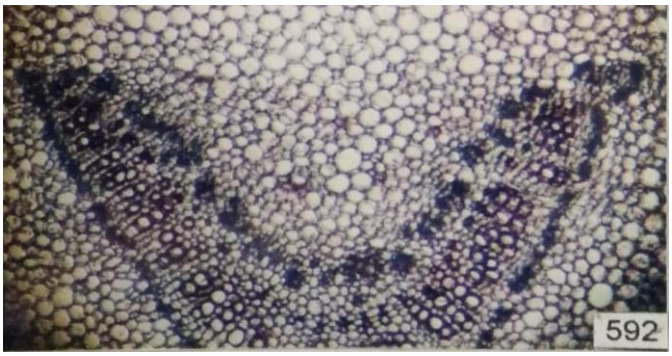

Fig.3 (592): Transections of leaf mdrib. (X40)

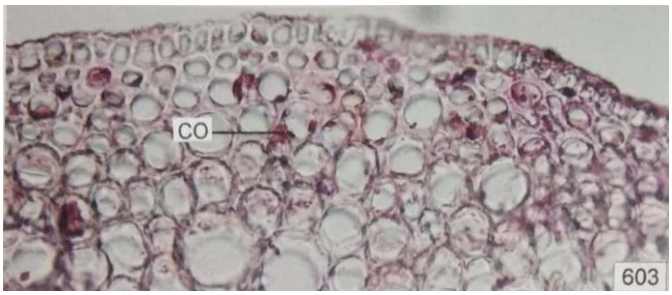

Fig.4 (603): Transections of midrib of leaf. (X120, CO- collenchyma).

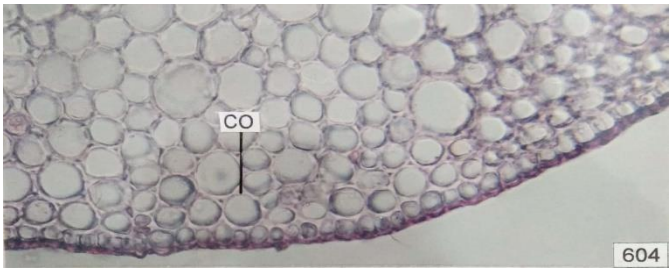

Fig.5 (604): Transections of midrib of leaf. (X120, CO- collenchyma).

The adaxial and abaxial epidermis of the cotyledons are single layered, cells are tubular, squarish or rectangular and arranged without intercellular spaces. Stomata and trichomes are present in the epidermis. The outer surface of the epidermis is cuticle. The cuticle is normally thin. The adaxial epidermal cells are larger than the abaxial ones in *Adenium obesum* (Figs. 8(622) The wall of epidermal cells may be sinuous or non -sinuous on both surface

or sinuous on one and non -sinuous on another surface.

2.7 Anatomy of Cotyledon Mesophyll

The mesophyll is differentiated into palisade and spongy tissue normally. But in *Adenium obesum* mesophyll is differentiated into palisade and chlorenchyma (Figs. 8(622). The palisade cells are relatively shorter and oriented perpendicular to the adaxial surface in one to three rows (Table 2). The cotyledons of investigated specie are dorsiventral since the palisade cells are located to adaxial side and spongy parenchyma or chlorenchyma to other side.

2.8 Anatomy of Cotyledon Vascular system

Since the cotyledons have pinnate venation, smaller vascular bundles cut in transverse, oblique or longitudinal planes are observed in lamina (Figs. 8(622), They are few, small, mostly collateral with adaxial xylem and abaxial phloem and not associated with supporting cells or well organized sheath.

2.9 Anatomy of Cotyledon Midrib Epidermis and ground tissue

The structure of epidermis and cuticle of midrib is similar to lamina Midrib ground tissue is
Paranchymatous. These cells are larger circular or oval, thin with intercellular spaces. Hypodermal chlorenchyma are continuous with the palisade layer of lamina.

2.10 Anatomy of Cotyledon Vascular system

Since the cotyledons have pinnate venation. There is a thickest vein along the median longitudinal axis. This can be observed as a central vascular bundle in transactions. It is conjoint collateral or bicollateral and crescentic in most of the species except *Adenium obesum* were midrib bundle is much smaller in size; oval or roundish in shape and surrounded by ill — defined sheath of parenchyma (Figs. 9(635).

2.11 Anatomy of Cotyledon Petiole

The petiolate leaves are present in most of the species of Apocynaceae. There is very small petiole present in *Adenium obesum*.

2.12 Anatomy of Cotyledon Epidermis and ground tissue

The epidermis is unilayered of rectangular or squarish or tubular cells. The outer surface is cutinised. The ground tissue is parenchymatous, cells oval or roundish with intercellular spaces. The peripheral cells of ground tissue may contain chloroplasts.

2.13 Anatomy of Cotyledon Vascular system

It consists of one bundle or a pair of bundles depending upon the region of petiole. The presence of one bundle may be due to the approximation and ultimate fusion of the two bundles. Petiole bundle /bundles are collateral or bicollateral.

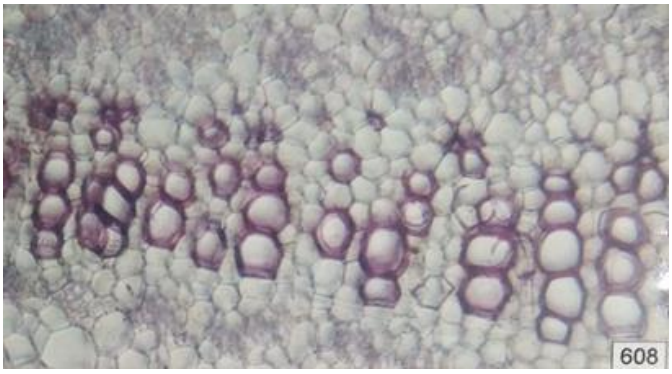

Fig.6 (608): Transections of leaf midrib. (X120).

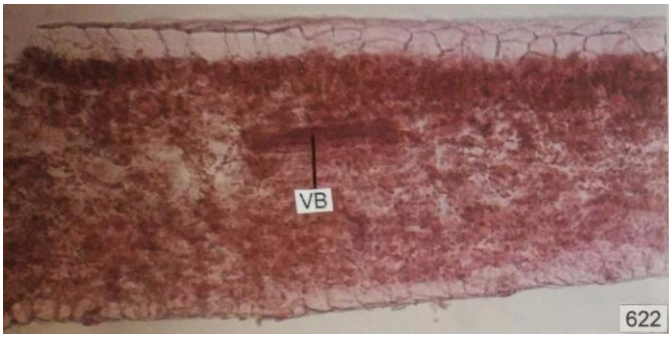

Fig.7 (622): Transections of cotyledon. (X120, VB-vascular bundle)

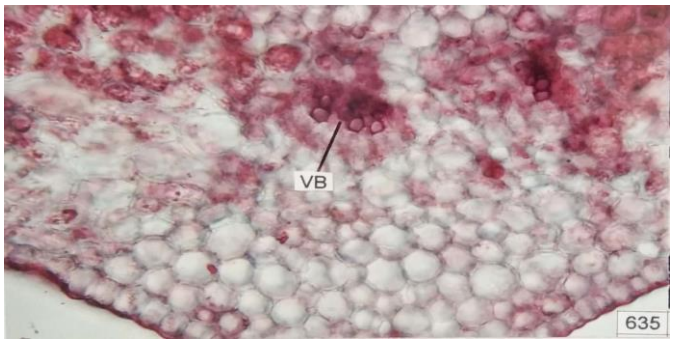

Fig.8 (635): Transections of cotyledon. (X120, VB-vascular bundle)

IV. CONCLUSION

The seedling of *Adenium obesum* differs by following morphological features from the other seedlings of Apocynaceae: (1) Late sprouting and stunted growth (2) Short, sturdy, flexuous roots (3) Short, fleshy, thick, barrel shape (Swollen from middle and narrow from ends), soft, slow growing hypocotyl, (4) Slow growing, soft, fleshy, thick epicotyl and (5) Smaller succulent cotyledons. These characters are useful in taxonomic and systematic studies of the family.

In secondary xylem of *Adenium obesum* the VES with pitted thickenings occur in higher frequency. This is phylogenetically advance character as highly evolved VES have pitted thickening (Bailey, 1953; Bierhost, 1960). Though reticulate and scaleriform types are somewhat not common, it is interesting to note that their frequency is relatively higher in hypocotyl and epicotyl of *Adenium obesum*. VES of the organs of Adenium obesum differ from the seedlings of other species and can be regarded as evolutionary primitive elements.

In the present study for the VES of Adenium obesum the combination of two different types of wall thickenings in a same VE are observed. They are reticulate - pitted, scaleriform - pitted and reticulate - scaleriform wall thickenings. Such combinations are more common in VES of *Adenium obesum* and can be considered as evolutionary transitional forms.

In *Adenium obesum*, the cotyledonary node as well as leaf nodes are unilacular with five traces departing from the vascular system of seedling axis, i.e. it is a multitrace unilacular nodal type. This condition has been reported earlier in cotyledonary nodes (Kato, 1966; Sugiayama, 1970) and in adult leaves with unilacular node (Watari,1934, 1936, 1939; Benzing 1967; Kato 1967). This condition is not frequent in species with unilacunar nodes (Sinnott, 1914; Philipson and Philipson, 1968; Dickison, 1975; Neubaner, 1981).

In the present work, isolated veins are not observed but isolated trachieds are found in the mesophyll of *Adenium obesurn*. The hypocotyl of *Adenium obesum* differs from other species of Apocynaceae as it is short, fleshy and slow growing. The vascular system in all species is formed by small vascular bundles. They are found in a ring and are present in different number in different species. The maximum number of strands are found in *Adenium obesum*.

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