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Leaf and Cotyledon Architecture in The Seedling of *Catharanthus Roseus* (L.)

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ABSTRACT

Catharanthus roseus is a perennial small herb or sub-shrub, up to 90 cm in height. Stem is erect, with flexible long branches, purple or light green. Leaf architecture and cotyledon architecture including venation pattern has been studied in *Catharanthus roseus* (L.) of the Apocynaceae. The Leaves are simple, cauline, opposite, ex-stipulate, petiolate, elliptic ovate to oblong, 4-10 by 2-4 cm glabrous to pubescent, base acute or connate, apex obtusely apiculate and lateral nerves 10-12 pairs. Petiole is 1.0- 1.5 cm long. The major venation pattern conforms to pinnate camptodromous type with festooned brochidodromous secondaries. The qualitative and quantitative features are studied. The leaf and cotyledon size, areole size, number of vein endings entering the areoles and number of vein terminations entering the areoles vary from species to species even within the same species of *Catharanthus roseus*. The highest degree of vein order is observed up to 5°. Isolated tracheids, isolated vein endings, isolated free vein endings and tracheoidal elements are noticed. Loops may be formed by vein-lets only or by vein-lets and tracheids. The terminal tracheids of vein endings show an increase in cell diameter are of various shapes and occur single or in groups of 2 - 3 or more per vein-let terminus. The primary and secondary veins are surrounded by a multiseriate bundle sheath and vein-lets and vein endings have uniseriate sheath. Cotyledons have pinnate venation. They differ in sub type which is camptodromous, hypodromous or eucamptodromous. The primary, secondary and tertiary veins resemble in several characters. The marginal ultimate venation is incomplete or looped.

Keywords : Leaf, cotyledon, architecture, *Catharanthus roseus*

I. INTRODUCTION

Catharanthus roseus (synonymous with Vinca rosea) is a perennial plant commonly seen in tropical countries. Seven species of this genus are native to Madagascar and one species is native to Southern Asia. It is more commonly known as Madagascar periwinkle.(Sutarno and Rudjiman, 1999). The local name in Malaysia is Kemunting Cina. The National Cancer Council of Malaysia (Majlis Kanser Nasional, MAKNA) uses the periwinkle logo as its symbol of hope for cancer patients. This plant produces beautiful flowers with a variety of colors such as purple, pink and white and commonly planted for decorative purposes. Historically Madagascar periwinkle had been used for various treatments, e.g. diabetes mellitus, high blood pressure and infection. (Flora of China Editorial Committee,2015; PROTA,2015)

The stem of Madagascar periwinkle produced a milky sap which is the source of over 70 different indole alkaloids. Two of the common anti-cancer drugs which are derived from this plant are vincristine and vinblastine (they are named after Vinca). Vincristine is used in the chemotherapeutic regime for Hodgkin's lymphoma while vinblastine is used for childhood leukemia. These vinca alkaloid bind to tubulin dimers and inhibiting microtubule structures of the cells, thus inhibiting the metaphase of cellular mitosis. Their Main side effects of these drugs are peripheral neuropathy, hair loss, hyponatremia and constipation.

As Catharanthus roseus is very much important medicinal plants so its various anatomical aspects should be checked. Although flower and fruit characters have very useful in identification and delimitation of the genera and species, but sometimes its be a great need to study the vegetative characters also for study about medicinal plant and its pharmacognostical studies also.

Many researcher deals with many anatomical and histochemical aspects for the identification of true generic medicinal plants, but even there is very scanty work occurs in the study of leaf and cotyledon architecture.

Present paper is more focused on the detail study of leaf and cotyledon architecture of Catharanthus roseus at its seedling stage.

Table 1 : Morphological features of leaves

Texture	chartaceous
Symmetrical/ Asymmetrical	Symmetrical
Shape	Oblong
Apex	Acute
Base	Acute
Petiolate or sessile	Petiolate
Second pair of leaf phyllotaxy	Opposite dicussate

II. METHODS AND MATERIAL

Clearing

Fresh leaves and cotyledons were collected from the seedlings for the study of cotyledon and leaf architecture they were cleared by the method of Mohan Ram and Nayyar (1978) and stained with Safranin 0. The stained materials were mounted in 50% glycerin.

Leaf architectural characters

Leaf shape, venation, margin, base, apex, area, areolation, blade class, organization, presence of trichomes, and other epidermal (appendages/ indumentum) were examined following the standard and tested procedures (Dilcher, 1974; Hickey, 1973).

Photo microscopy

Photomicrographs were taken with a Carl-Zeiss photomicroscope using Agfa film. The photomicrographs of areole size, the number of vein lets entering in areole and the number of vein endings entering in areole were taken in five different fields of different leaves. Terminologies to describe leaf and cotyledonary architecture were adopted from Hickey (1973) and Hickey and Wolfe (1975).

III. RESULTS

1. Leaf Architecture

The organization of the first and subsequent leaf is simple consisting of a single lamina. Generally, the lamina and leaf base are symmetrical. The apex is short, obtuse, acute to attenuate, acute to obtuse or roundish and margin may be concave. The leaf base is obtuse, acute, cuneate or rounded. Generally, the leaf margin is entire and smooth without noticeable projections. The texture of leaf is chartaceous. Leaves are petiolate. Petiole is terrate. (Table 1).

2. Venation

2.1 Type of Venation

The venation is pinnate with a single primary vein(midvein). It is camptodromous as secondary veins do not terminate at margin. Hicky (1979) further classified the camptodromous venation into four types on the basis of the characters of secondary veins. In the leaves of the seedlings of Apocynaceae family subtype of venation is cladodromous as secondaries are ramified towards the margin orbrochidodromous. (Table 2).

Table : 2 Type of Venation

Type of venation	Pinnate
Subtype of venation	Camptodromous Cladodromous
Primary vein size	Massive
Course of primary vein	Straight unbranched
Secondary vein angle of divergence	Acute narrow
Marginal ultimate venation	Incomplete
Course of secondary vein	Sinuous smooth arc

2.2 Primary vein

The thickest vein is primary vein (Fig. 281). This serves as an origin for the higher order venation. Its thickness gradually decreases towards the apex. The size of primary vein is determined midway between the leaf apex and base as the ratio of vein width (Vw) to leaf width (Lw) (Hicky, 1979).

$$Size\ (Primary\ Vein) = \frac{Vw}{Lw} \times 100\ \%$$

Thus in dicotyledonous plants the size of primary vein can be:

(i) Massive :> 4.00 %
(ii) Stout: 2.00– 4.00 %
(iii) Moderate: 1.25 – 2.00 %
(iv) Weak :< 1.25 %

In the leaves of the seedlings the size of primary vein is “stout" or "massive" (Table: -2). The course of primary vein is straight (branched or unbranched) or sinuous (Table: - 2).

2.3 Secondary veins

The secondary veins (2°) arise on either side of the primary vein in alternate fashion (Fig. 275). The angle of secondary veins measured between the branch and the continuation of the source vein above the point of branching is acute moderate or acute narrow (Table 2). The course of secondary veins is curved bending in shallow or more or less straight arch.

The intersecondary veins are mostly thinner than secondaries (Figs. 275, 280, 281). Sometimes they are as thick as tertiary as in Catharanthus roseus. They originate from the primary vein and follow more or less a course similar to the secondary veins except Catharanthus roseus where they are at right angle to primary vein (Fig. 280, 281). They are simple or formed by the coalescence of the tertiary vein segments. In Catharanthus roseus intersecondary vein may join secondary veins (Fig. 281).

2.4 Tertiary veins

The finer branches of the secondary veins are tertiary veins (Fig. 281). They are ramified, branching into higher order with or without rejoining the secondary veins. They are wavy or straight. In Catharanthus roseus all tertiaries are produced at right angle and remain parallel and may join with opposite secondary or with the branches of secondary or may have blunt end.

2.5 Quaternary and quinary veins

The finest veins originating from tertiary are quaternary and those from quaternary are quinary veins. Hickey (1979) classified them in to two types;

(a) Thick: Wider than expected.
(b) Thin: narrow than expected.

The quaternary and quinary veins are distinct (Fig.281). They are classified as "thick" and "wide" and their course is relatively randomly oriented (Fig. 281).

The quinary veins are "thick" and their course is random similar to quaternaries.

2.6 The marginal ultimate venation

The marginal ultimate venation is incomplete with some freely ending vein-lets directly adjacent to the margin. The venation features are presented in table 2.

2.7 Vein-lets

The freely ending ultimate veins of the leaf is vein-lets. They are simple or branched The simple vein-let may be linear or curved. The vein-let in Catharanthus roseus has finger like projections at distal end.

The nature of the branched vein-let is interesting. Vein-let may terminate into two free branches The branching may be or unequal Out of the two branches (A, B) only one (branch B) again forms two branches The vein endings may be unisegmented, bisegmented or trisegmented in latter all the segments are uniseriate or sometimes the middle segment is biseriate The trachieds that terminate at the ultimate tips show anincrease in cell diameter. They may he present or in group of two) and three or more. In such cases their arrangement may be juxtaposed, superposed or juxtaposed – superposed. Sometimes juxtaposed trachieds are arranged near the end at right angle to multiseriate vein- let. They are of various shapes viz., roundish, pterygoid, vermiform, triangular, oval foot shape, cylindrical and barrel -shape.

2.8 Isolated Trachieds

An isolated trachieds are observed in Catharanthus roseus (Figs.281). They are not frequent.

2.9 Loop

Loop formation is observed in Catharanthus roseus. The loops may be formed by veinlets, tracheids or vein-lets and tracheids. It may be tetra angular, lenticular, pentangular in shape, occurring singly or in group of two.

2.10 Areoles

The areoles are the smallest areas of the leaf tissue surrounded by veins which form a contiguous field over most of the area or the leaf. They are angular in nut line, more or less variable in size and formed by all types of veins. Their arrangement is random showing no preferred orientation.

2.11 Vein sheath

In all the species investigated it is found that the primary vein is covered by a multiseriate bundle sheath, running parallel to the vein Fig. 281, Secondary also has multiseriate sheath. The vein-lets and vein endings have a uniseriate bundle sheath.

3. Cotyledon Architecture

In Apocynaceae seedling bears two opposite cotyledons with simple, symmetrical lamina (Table 3). Petiole may or may not be present but is always short and terrate or flat in petiolate cotyledons. The cotyledons are ovate, obovate, oblong and elliptic. The cotyledonary apex is short or minute, obtuse, acute, or rounded. The base is obtuse, obtuse to rounded, actute to obtuse, chordate or rounded (Table 3) Cotyledonary margin is entire, more or less concave and smooth without any type of projections.

Table 3: Morphological features of cotyledon

Lamina	Symmetrical/ Asymmetrical	Symmetrical
	Shape	Oblong
	Apex	Rounded
	Base	Acute to obtuse
	Photosynthetic/ Non-photosynthetic	Photosynthetic
Petiolate or sessile		Petiolate
Petiole		Flat

3.1 Venation

3.2 Type of Venation

The venation of cotyledon is pinnate with a single prominent primary vein. In *Catharanthus roseus it* is eucamptodromous where secondaries are upturned and gradually diminishing apically inside the margin (Fig. 342; Table 30)

3.3 Primary Vein

Primary vein or the midrib is the thickest of all the veins and occur single in all investigated species (Fig. 342). It traverses towards the tip without any branches. Generally, it is straight. Normally, the stout primary vein at the base gradually becomes lean towards the apex.

3.4 Secondary vein

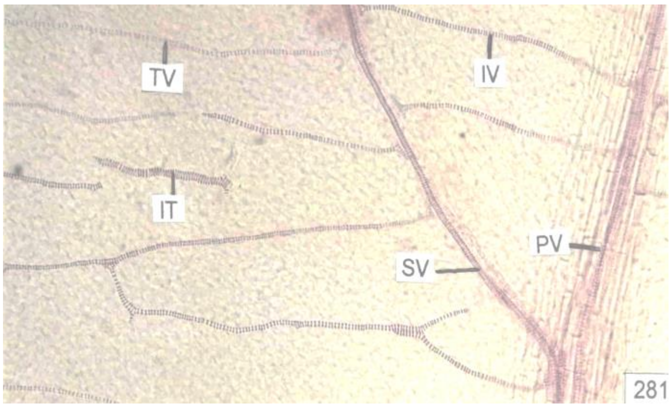
The arrangement of secondary veins on either side of the primary vein is alternate. Usually, the thickness of the secondary vein is more at the point of origin and decrease towards the margin. These secondary vein course may be straight or slightly curved or sinuous with repeated smooth or slightly angular changes in the direction of curvature. The angle of divergence of secondary veins is acute moderate or narrow. (Table 4). Branching of the secondary is observed in most of the investigated species. The branching is mostly dichotomous. The branches of two adjacent secondaries may unite. The intersecondary veins are relatively thinner than secondaries. They are produced from primary vein and follow more or less parallel course to the secondary veins. Intersecondary veins are not formed by the coalescence of the tertiary vein segments but, arc simple.

Table 4 : Venation features of cotyledon.

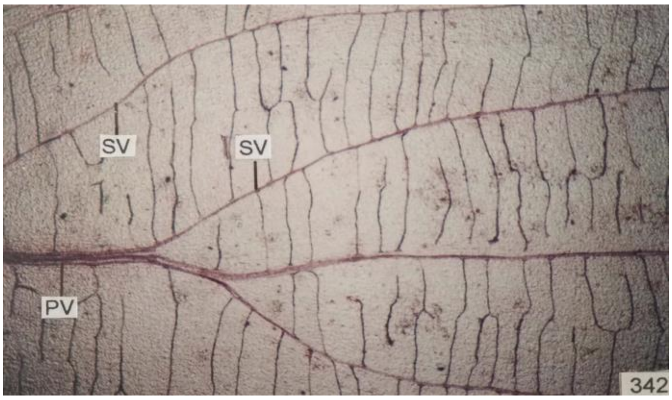
Plant	Catharanthus roseus
Type of venation	Pinnate
Subtype of venation	Camptodromous Eucamptodromous
Nature of primary vein	Grooved
Primary vein size	Stout
Course of primary vein	Zigzag
Secondary vein angle of divergence	Narrowly acute
Marginal ultimate venation	Incomplete
Course of secondary vein	Sinuous

3.5 Tertiary vein

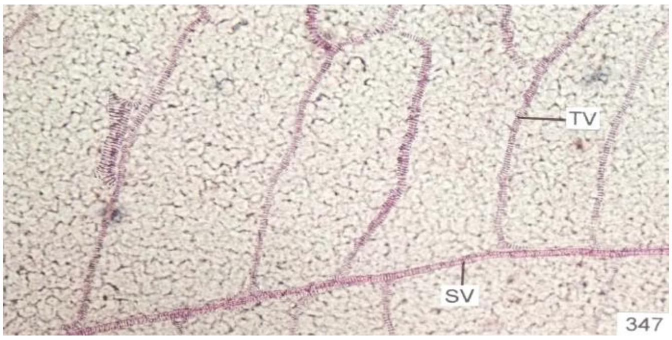
The next finer branches of the secondaries constitute the tertiary veins (Figs. 342, 347). Tertiaries are produced at right angle and remain parallel in *Catharanthus roseus* (Figs. 342, 347). These tertiary veins at times anastomose with other tertiary vein or secondaries or with thin branches and form orthogonal reticulate in *Catharanthus roseus* (Figs. 342, 347).



Figs. (281) : Surface view of the portion of cleared leaves of *Catharanthus roseus* (X120, IT- isolated tracheid ; IV- inter secondary vein ; PV – primary vein ; SV- secondary vein ; TV – tertiary vein) .



Figs. 342: Surface view of the portion of cleared leaves cotyledons of *Catharanthus roseus*. (X60, PV – primary vein; SV- secondary vein).



Figs. 347 : Surface view of the portion of cleared leaves cotyledons of *Catharanthus roseus* ; (X120, SV – secondary vein; TV – tertiary vein).

3.6. Marginal ultimate venation

In all the species investigated marginal ultimate venation is of two types incomplete and looped (Table 4). In the former type, the vein-lets end freely directly adjacent to the margin, found in most of the species investigated. In the latter type the ultimate venation recurves and forms loops towards the margin.

3.7 Quaternary and quaternary veins

The finest branches of tertiaries are quaternary veins and branches of quaternaries are quinary veins. Here only quaternary veins are studied. Following (Hickey, 1979) they are classified as "thick" and wider and their course is random oriented.

3.8 Areoles

The areoles are more or less angular in outline, variable in size and formed by all types of veins. They are imperfect with meshes of irregular shape. They show random arrangement with no preferred orientation.

3.9 Loops

Loops are not very common and observed in *Catharanthus roseus*. They are formed by veins and tracheids or by tracheids alone. Their shape may be lenticular triangular rectangular or polygonal. They occur singly or in groups of two.

3.10 Veinlets

The veinlets are simple or branched. The simple vein-let may be linear or curved. The vein-let branching is more or less similar to leaf. A vein-let divides to form two branches which are equal or unequal. Out of the two newly produced branches (A, B) any one divides to form two free branches. The vein ending may be unisegmented, bisegmented or trisegmented. The tracheids situated on the ultimate tips are broad with larger diameter. They are present singly or in groups of two, three or more. Their arrangement may be juxtaposed, superposed, juxtaposed-superposed or irregular. These tracheids exhibit variation in their shape. They may be cylindrical Conical, triangular.

3.11 Vein Sheath

The primary vein covered by a multiseriate bundle sheath running parallel to the vein. Secondaries may also be covered by multiseriate sheath. The veinlets and vein endings have uniseriate sheath.

IV. DISCUSSION

1. Leaf Architecture

The most comprehensive surveys of the venation of foliage leaves was carried out by Von Ettinghausen (1854a, 1854b, 1957, 1858a, 1858b, 1861, 1865, 1872). This classical study, although devoid of morphological and phylogenetic conclusion, nevertheless represents the first attempt to devise a classical descriptive classification of venation systems based on the course of main veins in the lamina. From the standpoint of venation type the classical studies of Von Ettinghausen (1861) remained the most extensive to date and had greatly influenced the later classifications (de Bary, 1884; Kerner and Oliver, 1894). Melville (1969) traced the evolution of the angiospermic leaf from Permian *Glossopteris* by comparing the venation pattern of living plants from the countries which formed Gondwanl and with their fossil records. Hicky (1973) developed a classification on leaf architecture in dicotyledonous leaves. Melville (1976) gave terminology of leaf architecture of angiosperms. Foster (1950) in his study on venetation of *Quiina acutangula,* explained the need for intensive exploration of leaf venation. Perhaps, his opinion might have prompted many workers to undertake such studies and in the last several years, considerable work has been done on leaf venation in angiospermous plants. The leaf architecture in the family Apocynaceae had not been studied except the work of Chandra *et al.* (1972).

The study of leaf architecture of the seedlings of *Catharanthus roseus* of Apocynaceae reveals that they resemble in following features in venation pattern:

1. Venation is pinnate, camptodromous.
2. Primary vein is "stout" or "massive" and its course is straight or sinuous.
3. The course of secondary veins is curved bending in shallow / more or less straight arch.
4. Inter secondary veins are observed.
5. Teritary veins straight and per current joining with other tertiary veins of opposite secondaries.
6. Quaternary and Quinternary veins are "thick".
7. The marginal ultimate venation is "incomplete" or "looped".
8. The arrangement of moles is random.

The present study also shows different types of vein-lets, the branching may be once or twice, equal or unequal. The vein endings may be unisegmented, bisegmented or trisegmented with uni or biseriate segments.

Pray (1954) found that the terminal tracheids of vein endings show an increase in cell diameter. Such terminal tracheids are common in the leaves of the seedlings of investigated species. They are also reported in the leaves of Papilionaceae (Rao, 1981; Patel, 1999) and Caesalpinaceae (Shah, 2001).

The tracheids are variously shaped, occurring singly or in groups of 2 — 3 or more per vein-let terminus. According to Strains (1933) vein endings are of taxonomic significance but Dckison (1969) believes them to be of limited importance. On the other hand, Hall and Melville (1951, 1954) and Varghese (1969) painted out that they can be used at taxonomic levels, in the conjunction with other characters. From the present study it can be suggested that they are of less taxonomic value since more than one type are observed in almost all the investigated taxa.

It seems Kasapligil (1951) was the first to report the occurrence of isolated veins (cf. Herbst, 1972). He is followed by Foster (1966, 1968), Foster and Arnott (1960), Lersten (1965), Herbst (1972) and Inamdar and Murthy (1978) in recording their presence in _ several dicotyledonous plants. According to Hara (1962) such isolated veins may be formed when certain extension cells fail to differentiate into vascular tissue. In the present work, isolated veins are not observed but isolated trachieds are found in the mesophyll of *Catharanthus roseus.*

The primary and secondary veins are surrounded by a multiseriate bundle sheath and vein-lets and vein endings have uniseriate bundle sheath. Wylie (1952) studied the bundle sheath of several dicotyledonous plants and found that bundle sheath cells are closely joined and intercellular spaces are not farmed. Rao (1981) observed intercellular spaces arising schizogenously in the contiguous wall of the sheath cells in *Trifolium dubium.* Such schizogenous intercellular spaces are not observed in the leaves of Catharanthus *roseus* of Apocynaceae.

2. Cotyledon architecture

The study on the cotyledon architecture is scanty dicotyledon. Rao (1981), Gupta (1978) and Patel (1999) studied the cotyledonary architecture in Papilionaceae. Shah (2001) carried out such study in Caesalpiniaceae. As far as author is aware cotyledon architecture has been first time studied in Apocynaceae.

The cotyledons of investigated species resemble in main venation type which is pinnate. Pinnate venation may be camptodromous, hypodromous or eucamptodromous. The cotyledons are symmetrical, elliptic, obovate, ovate or oblong with obtuse, acute or rounded apex and cordate, rounded, acute to obtuse, obtuse to rounded base are observed. They are photosynthetic in *Catharanthus roseus*. There are several characters of primary vein viz.; number, nature in adaxial surface and course in cotyledon etc. are similar in most of the taxa. The angle of divergence of secondary vein is narrow — acute or moderate — acute and it has straight course. The loops are of various shapes. It is formed by veinlets or trancheids or by both. Such loops are also observed in the cotyledons of Papilionaceae (Rao, 1981; Patel 1999) and Caesalpiniaceae (Shah 2001).

The vein lets may be simple or branched. Branching resemble to leaf vein lets. The vein endings may be unisegmented bisegmented or trisegmented. The tracheids situated on the ultimate tips of vein-lets are larger in diameter and resemble to leaf tracheids in number in groups, arrangement and shape.

V. CONCLUSION

Based on result obtained in Catharanthus roseus there are very unique pattern found in Leaf architecture, where primary vein is thick and straight. They are pinnate, camptodromous venation. Tertiary venations are parallel to each other, which is the unique pattern in both leaf and cotyledons. Tertiary vein-lets are united with each other and produce loop in-between. Cotyledons have pinnate venation. They differ in subtype which is camptodromous and eucamptodromous.

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