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Seedling morphology: an effective tool for the identification of South Indian species of Rauvolfia (Apocynaceae)

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Abstract **- The study focused on the morphological characteristics of seedlings, with a particular emphasis on the cotyledons and eophyll features of five *Rauvolfia* species: *Rauvolfia hookeri* Srinivas. et Chithra, *Rauvolfia micrantha* Hook.f., *Rauvolfia serpentina* (L.) Benth. ex Kurz, *Rauvolfia tetraphylla* L., and *Rauvolfia verticillata* (Lour) Baill. The research involved documenting these characteristics. Based on the documented seedling traits, such as the shape, size, and venation of cotyledons and eophylls, a key for identifying *Rauvolfia* seedlings was proposed. The observations highlighted differences among all the species, with distinct characteristics, while some common features were also noted. These findings are valuable for both ex situ and in situ conservation efforts of these species.**

Keywords**: - Cotyledon; Eophyll; Kladododromous; Paracotyledon; Venation.**

I. INTRODUCTION

The *Rauvolfia* genus, which falls under the Apocynaceae family, comprises approximately 110 shrub species, primarily found in tropical regions (Mabberley, 1990). India is home to five of these species, namely *Rauvolfia hookeri* Srinivas. et. Chithra, *R. micrantha* Hook. f., *R. serpentina* (L.) Benth. ex Kurz, *R. tetraphylla* L., and *R. verticillata* (Lour.) Baill. (Gamble, 1921). Notably, *R.*

hookeri and *R. micrantha* are exclusive to the southern Western Ghats (Ahmedullah and Nayar, 1986). The roots of *R. serpentina* are utilized to extract 'serpentine,' a crucial medication for hypertension treatment (Anonymous, 1969). Other *Rauvolfia* species also contain substantial alkaloid quantities, serving as potential substitutes for *R. serpentina* (Bindu, et al., 2014). However, excessive harvesting and habitat destruction have resulted in the rarity of these species in their natural environments (Nayar, 1996). Ahmedulla and Nayar (1986) classified *R. hookeri* as a threatened species. Singh (1999) included *R. serpentina* in the list of plants that are either on the brink of extinction or have become rare in India. The Conservation Assessment and Management Plant listed *R. serpentina* as a critically endangered plant (Kunwar, 2019).

Seed germination in *Rauvolfia* species typically begins with the emergence of the radicle within a span of 28 to 32 days, followed by the elongation of the plumule. A seedling consists of two parts: the portion above the cotyledon attachment is the epicotyl, and the portion below the cotyledon is the hypocotyl. As the hypocotyl extends, the cotyledons rise above the soil in an epigeal germination. Initially, cryptogamous cotyledons are non-photosynthetic, but during the progress of epigeal germination, they become photosynthetically active, transforming into phanerocotylar cotyledons.

Paracotyledons are photosynthetic but differ in structure from foliar leaves, as they do not expand or possess veins (De-Vogel, 1980). According to Garwood (1996), photosynthetic cotyledons offer advantages for small seeds in high-light environments, while reserve cotyledons can support seedlings in low-light, stressful conditions.

Based on germination characteristics and cotyledon positioning, seedlings are classified as either epigeal (cotyledons are raised above the soil, supported by an elongated hypocotyl and exposed to light) or hypogeal (cotyledons are positioned at soil level, typically enclosed within the fruit wall and/or testa). De Vogel (1980) categorized seedlings based on their development. Cotyledons can either remain enclosed within the testa (cryptocotylar) or emerge from it (phanerocotylar). Cotyledons can also serve as reserve organs (assimilating cotyledons) or foliaceous, photosynthetic structures (paracotyledons).

While the morphology of seedlings in mangrove species has been extensively studied, providing critical information for their identification (Shaikh, et al., 2010), seedling morphology in *Rauvolfia* species remains unexplored. Reports have documented the occurrence of polyembryony and the establishment of such seedlings in *R. serpentina* (Bindu, et al., 2007). Singh (2015) conducted a taxonomic study of the morphology of some dicot weed seedlings to aid in their identification. This study focuses on the seedling morphology of five *Rauvolfia* species, aiming to facilitate their conservation through nursery or homestead cultivation strategies.

II. MATERIALS AND METHODS

Seeds obtained from plants grown in identical conditions were placed in a seed germinator, using moist acid-free paper towels for incubation. Once the radicles emerged from the seed coat, they were transplanted into plastic trays filled with a mixture of sand and topsoil to facilitate further growth. All species were maintained under consistent shaded conditions. At each leaf stage, starting from cotyledons, data was systematically gathered for characteristics including hypocotyl length, epicotyl length, cotyledon length and width, eophyll length and width, cotyledon/leaf shape, petiole length, internode length, and cotyledon/leaf arrangement, with five seedlings measured for each species. Growth coefficients, relative growth rates, stem-weight ratios, seedling length vigour indices, and seedling weight vigour indices were computed based on the collected data, employing the formulae outlined by Khurana and Singh (2000). The terminology used adhered to the definitions established by De Vogel (1980).

III. RESULTS

The cotyledons consistently appear in pairs, positioned opposite each other, emerging by splitting the two layers of the seed coat. They initially expand to take on a thin, leaf-like structure, starting with a yellowish hue that gradually turns green upon exposure to light. The persistence of cotyledons was noted to be unique to each species. The shape of cotyledon apices was observed as either obtuse or acute, and the venation displayed either kladodromous or brochidodromous patterns. The data collected clearly demonstrates the presence of species-specific traits among all the species under investigation. The compiled data is presented below.

Rauvolfia hookeri Srinivas. et Chithra

The seedlings exhibit phanerocotylar development with an epigeal, Macaranga-type germination. They establish a robust taproot system and initially feature a curved hypocotyl resembling a hook during early emergence, which later straightens out (as seen in figure 1). The basal portion remains slightly curved, appearing whitish green towards the upper part and white at the base. At the cotyledon stage, the hypocotyl measures 26mm, while at the 4th leaf stage, it measures 38±1.08mm.

The paracotyledons are green with a yellowish marginal line, possessing a glossy, ovate shape with an obtuse apex and an attenuated base. They measure 14.04±1.02 x 11.36±0.23mm during the cotyledon stage. The petiole is short, reaching a length of up to 2mm, and the surface is smooth (as seen in figure 2a). The eophylls feature acuminate apices and attenuated bases, with brochidodromous venation.

Additionally, six pairs of alternate secondary veins were observed, demonstrating kladodromous branching near the leaf margin. The testa remains attached to one of the cotyledons until the 1st eophyll stage.

Rauvolfia micrantha Hook. f.

The seedlings exhibit phanerocotylar growth, characterized by epigeal germination of the Macaranga type. They establish a robust taproot system, with initial root growth being horizontal before transitioning to a vertical downward direction (as shown in figure 1). Lateral branches are abundant and appear white. The testa, or seed coat, is naturally shed after two weeks of sprouting.

The hypocotyl appears whitish green, measuring 40.88 mm at the cotyledon stage and 52.75±1.03 mm at the 3rd leaf stage. The cotyledons, also known as paracotyledons, are green and petiolate. During the cotyledon stage, they measure 2 mm, featuring oval-shaped, glabrous blades that are 17.63±1.41 mm long and 10.13±0.66 mm wide. The cotyledons have an obtuse apex and an attenuated base (as depicted in figure 2b). The margins are smooth, and the venation pattern is kladodromous, with secondary veins branching towards the ends. There are five pairs of alternate secondary veins, and the surface texture is smooth and glabrous. Paracotyledons persist throughout the seedling's growth period.

In the eophylls, an acuminate apex and an attenuated base are observed, with kladodromous venation, similar to that of the cotyledons.

Rauvolfia serpentina (L.) Benth. ex Kurz

The seedlings exhibit phanerocotylar growth, featuring an epigeal germination of the Macaranga type. They have a robust taproot system and a healthy appearance. The hypocotyl is straight and whitish, measuring 56mm at the cotyledon stage and 64mm at the leafy stage (as seen in figure 1).

The paracotyledons are green, equal in size, and positioned opposite each other. They are dark green, ovate in shape, with an obtuse apex and an attenuated base. These glossy paracotyledons measure 14.88±0.13 mm in length and 9.08±0.05 mm in width during the cotyledon stage, with a short petiole measuring 4 mm in length. The primary vein is straight, and there are five pairs of secondary veins with a kladodromous venation pattern (as illustrated in figure 2c).

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The testa separates to reveal yellowish cotyledons shortly after emergence, which soon turn green. These cotyledons persist until the end of the 5th leaf stage. Eophylls feature an acute apex and an attenuated base, with brochidodromous venation, where secondary veins do not merge near the leaf margin.

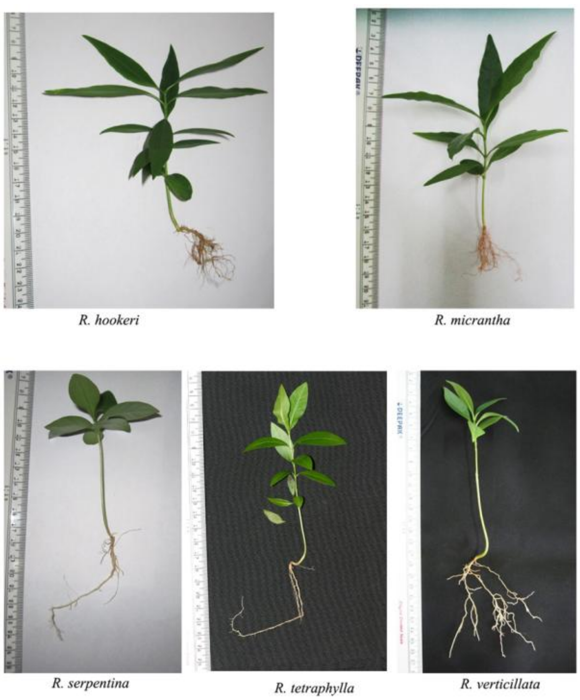

Figure 1: Seedlings of *Rauvolfia* species showing the root system.

Rauvolfia tetraphylla L.

The seedlings follow a phanerocotylar growth pattern, demonstrating epigeal germination of the Macaranga type. They possess a healthy taproot system. The base of the hypocotyl exhibits a slight curvature, which persists (as depicted in figure 1). The hypocotyl is whitish and gradually transitions to green as it extends upwards, measuring 57 mm during the 1st and 2nd leaf stages.

The paracotyledons are light green, ovate in shape, with an acute apex and an attenuated base. They have a short petiole measuring 1.75 mm, with dimensions of 16.55±0.05 mm in length and 7±0 mm in width during the cotyledon stage. The primary vein is straight, and there are six pairs of secondary veins, exhibiting a kladodromous pattern (as seen in figure 2d).

The seed coats naturally wither away between the 10th and 15th days, freeing the cotyledons. Minute hairs are present on both surfaces of the hypocotyl and leaf lamina. The mid-vein and lateral veins become less distinct towards the tips. In most seedlings, the cotyledons wither off during the 4th or 5th leaf stage. The epicotyl is short, reaching up to 12 mm. Eophylls display an acute apex and an attenuated base, with kladodromous venation.

Rauvolfia verticillata (Lour.) Baill.

The seedlings exhibit phanerocotylar growth with epigeal germination of the Macaranga type. They have a robust taproot system. The hypocotyls are notable for their length, measuring 84 mm, and they are swollen with a diameter of 2.23 mm. The basal part of the hypocotyl is curved, appearing white, while the upper section is dark green (as depicted in figure 1).

The paracotyledons are dark green, equal in size, and oppositely positioned. They have an oval shape, with an acute apex and an attenuated base. The leaf blades are smooth, with an entire margin. During the cotyledon stage, they measure 15.25±0.25 mm in length and 7.63±0.08 mm in width. They are petiolate, with a petiole length of 5 mm. These paracotyledons shed their seed coats approximately 20 days after emergence and fall off after the 2nd leaf stage, which occurs around the 76th day after emergence.

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The primary vein is prominent and clearly visible on both sides, and there are six pairs of secondary veins displaying a brochidodromous pattern (as shown in figure 2e). The eophylls have a narrowly acute apex and an attenuated base, with brochidodromous venation, akin to the paracotyledons.

Figure 2: Cotyledons of *Rauvolfia* species with embryo.

IV. DISCUSSION

Cotyledons serve as a crucial basis for classifying plants into dicotyledons and monocotyledons. Even within the same family, there exists species-specific diversity, as seen in the Apocynaceae family where cotyledons can take various forms such as ovate, obovate, oblong, and elliptic. Their margins may be entirely smooth, their apex obtuse, acute, or rounded, and their bases may vary from obtuse, rounded, acute, to even chordate (as observed by Kailash in 2013).

In the case of *Rauvolfia* species, it was noted that all species have isocotylar germination, meaning they possess two equally sized cotyledons. These cotyledons are oval in shape, featuring an obtuse or rounded apex. However, in *R. tetraphylla* and *R. verticillata*, the apex is acute. *R. hookeri* exhibited the largest cotyledonary leaf area. Additionally, *R. tetraphylla* displayed pubescent characteristics in its adult stage. Some of these traits were found to correlate with features in adult plants, particularly in certain timber-yielding species, as observed by Saha and colleagues in 1998.

For all species, the shape of the leaves was elliptic, except for *R. verticillata*, which had an ovate leaf shape. Similarly, the leaf apex shape was acute in *R. tetraphylla* and *R. verticillata*, while in the other species, it was attenuate. Anilkumar et al., (2011) studied the seed morphology of *Rauvolfia* species. Mohan and Inamdar's 1982 study of Apocynaceae leaf architecture described *Rauvolfia serpentina*'s leaf shape as ovate. The venation of paracotyledons and eophylls could be either similar or dissimilar among species. In *R. micrantha* and *R. tetraphylla*, both paracotyledons and eophylls exhibited kladodromous venation, while in *R. verticillata*, they displayed brochidodromous venation in both types of leaves. *R. hookeri* and *R. serpentina* had kladodromous venation in cotyledons and brochidodromous venation in eophylls.

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The study of seedlings is considered one of the most essential methods for identifying plant species, as noted by Kumar and Chauhan in 2016. Based on the characteristics described for *Rauvolfia* seedlings, a key for identifying these species in the seedling stage has been proposed.

Key for seedling identification

1. Cotyledons acute at apex ………………….2
1. Cotyledons obtuse at apex …………………3
2. Leaves with kladodromous venation……………………… *R. tetraphylla*
2. Leaves with brochidodromous venation…………………... *R. verticillata*
3. Leaves with brochidodromous venation…... 4
3. Leaves with kladodromous venation……………………... *R. micrantha*
4. Cotyledon's secondary veins 6 pairs……………………... *R. hookeri*
4. Cotyledons's secondary veins 5 pairs…………………..... *R. serpentina*

V. CONCLUSION

A classification key has been introduced to aid in the identification of *Rauvolfia* genus seedlings. This proposed key categorizes the seedlings into two distinct groups. In the first group, two species, *R. tetraphylla* and *R. verticillata*, share obtuse cotyledon apices, although they differ in eophyll venation patterns. In the second group, which also features obtuse apex cotyledons, *R. micrantha* is distinguished from *R. hookeri* and R. serpentina due to their varying leaf venation types.

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