**Anatomical Adaptations in Roots of Four *Nervilia* Species (Orchidaceae).**

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**ABSTRACT**

The present study analyzed the characteristics in roots of Terrestrial Orchidaceae member i.e four different species of *Nervilia* these are ***Nervilia aragoana* Gaudich** ,***Nervilia infundibulifolia* Blatt and Mc Cann**, ***Nervilia crociformis* Zoll and Moritzi Seidenf., *Nervilia plicata* Andrews Schtr**, explores the anatomical characteristics from an ecological point of view. The anatomical characters have served as a tool for the genus systematic. The plants were collected at different regions from Shivamogga district includes different forest types. Materials were fixed in FAA solution. Transverse sections were obtained by freehand sections. Observe the anatomical characteristics in four different species of *Nervilia* and analyze the anatomical adaptations in four different species. The prominent characteristics of the terrestrial *Nervilia* group presented simple and uniseriate epidermis and endodermis with less developed Casparian strips. Cortex cells have simple parenchymatous and filled with mycorrhiza association. Vascular cylinders number varied among four species and xylem phloem were easily distinguishable. Anatomical characters were common in four different *Nervilia* species, have evolved with the number of adaptations to different environmental conditions these adaptations help for *Nervilia* species to survive in a different type of vegetation with different environmental condition.

**KEYWORDS:** Orchidaceae**,** Anatomical character,Terrestrial Orchids, Adaptations.

**INTRODUCTION**

The Orchidaceae is one of the largest and widespread families of flowering plants, with highly evolved and specialized plant character. The Orchidaceae have about 25,000-35,000 currently accepted species, distributed in about 600-800 genera (Arditti,1977; Dressler,1981). Eastern Himalaya and the Western Ghats are the richest Orchid habitats in India. Recent researches show about 1300 species distributed in 151 genera in India (Medhi *et .al*.2012). According (Rao 1998) more than 46% of the known species of orchids are endemic to the Western Ghats. In Karnataka Orchidaceae family represented by about 203 species and 59 genera (Krishnaswamy *et.al.,*2004 a). Although its flowers present a relatively uniform structure, the organization of vegetative parts are notably variable among species (Dressler,1993).,such features allow the family to thrive in different environments and increase the number of its different grow forms (Pabst & Dungs,1975). During the evolutionary process, the orchids have become adapted to distinct environments, so they can be classified into epiphytes, terrestrial, saprophytes or lithophytes (Black, 1973 ). These environmental variations contribute to structural alternations in vegetative organs (Pabst and Dungs,1975 ). The terrestrial species absorb their nutrients directly from the soil and may present three kinds of roots:1)one adapted to absorption and fixation. 2) Tuber adapted to nutritive substance, storage and 3) One specialized in storage, absorption and fixation (Black, 1973). According to Dressler,1993 the tubers can survive during the dry season and nourish the plant when it is necessary.

*Nervilia* is commonly known as shield orchids. Plants were widely distributed across most of the areas. Plants are terrestrial, perennial, deciduous, sympodial herbs with an oval to almost spherical tuber have some short adventitious roots. One or two flowers are borne on an erect, fleshy, leafless flowering stem. When flowering the plants lack leaves but a single erect or ground-hugging leaf develops after the flower has fully opened. The leaves are usually wrinkled or crumpled with distinct, fan-like veins. The flowers are often short-lived, lasting for only a few days. The sepals and petals are similar, but the labellum is prominent and often composed of three lobes. Identification of genus *Nervilia* is easy when this species is in leaves, four different species of *Nervilia* are identified with the help of their leaf characters (Satish Pande *et.al,.*2010). ***Nervilia aragoana*** **Gaudich.** Petiole long, leaf blades not lying flat on the ground. ***Nervilia infundibulifolia* Blatt &Mc Cann**, leaf blade lying flat on the ground, petiole are 3-6cm long after the maturation, locally common in shade, Leaf blade green colour, chocolate coloured bands along the nerves are present when leaves are young. ***Nervilia cruciformis*** **(Zoll & Moritzi) Seidenf.** Leaf-blade green, petiole grows up to 6cm long, leaf blade flat on the ground, hairs are present on the upper surface. ***Nervilia plicata*** **(Andrews)Schtr**. Leaf-blade purple with brown hairs, shape varies from oval to cordate. The Leaf has short petiole, Occasionally green patches are also present on leaves.

**MORPHOLOGY**

***Nervilia crociformis*** **(Zoll & Moritzi)Seidenf.**

Commonly known as the trembling *Nervilia* or round shield orchid. Plants are common in shades of large trees, tubers fleshy globose or ovoid, corm like, with a few short roots at nodes or rootless. Stem arising from apex of tuber and giving rise to an inflorescence and a leaf in succession. Leaf hairy, cordate, flat on the ground. Flowers are solitary, sepals green, petals yellow, lip purple 3-lobed lateral lobes are long as mid lobe rounded and fimbriate, flowers are in April, leaves are formed after the flowering in August.

***Nervilia infundibulifolia* Blatt & Mc Cann.**

Commonly known as the Funnel leaf *Nervilia*. It is a small-sized terrestrial orchid with a small-sized tuber. The tuber produces tall straight stem with short internodes. Leaves are broadly heart-shaped to funnel-shaped with folded margins, gradually narrowing below into the base. The plant blooms from June to July month, on an erect, single flowered inflorescence. Flower arises singly on a leafless stem flower is 2-4 cm across.

***Nervilia plicata*** **(Andrews)Schtr.**

Terrestrial orchid is characterized by a single leaf. The Leaf is a prostrate, petiolate, ovate to rounded at base, margin entire or wavy, nerves fan-the like, the leaf appears after the flowering, inflorescence 1-3 flowered, Leaf blade purple with brown hairs, Occasionally green patches are also present on leaves. Flowering in May-June.

***Nervilia aragoana* Gaudich.**

It is a peculiar terrestrial orchid, Leaf broad, petiolate, acute at the tip. Tubers are small, producing long slender petiole, the tuber has 3-4 adventitious roots. Inflorescence 2-5 flowered, flowers are 2-5cm long greenish-yellow in colour. Plants commonly in shades, blooms between January to April, leaves in July-August.

Orchid roots do not derive nourishment from host plants hence orchids are not parasites, the important functions that orchid roots perform anchorage in the substratum and absorption of water and minerals.

*Nervilia* species has tubers with 3-4 absorption roots. The research explores anatomical characteristics in absorption roots of four *Nervilia* species and analyzing the characteristics concerning with environmental conditions.

**MATERIALS AND METHODS**

Shivamogga district is selected as the study area. Shivamogga is a district which is located in the Karnataka state of India. A major part of Shivamogga district lies in the Malnad region of the Western Ghats. There are seven taluks. Bhadravati, Hosanagara, Sagara, Shivamogga, Shikaripura, Soraba and Thirthahalli. The Terrestrial Orchids ***N.crociformis, N.plicata, N.infundibulifolia, N.aragoana,*** were collected at four different regions from Shivamogga district at different vegetation types includes evergreen forest, deciduous forest and open forest type. To evaluate the root anatomy, samples were fixed in FAA 50% for 48 hours and then transferred to Ethanol 50% (Johansen,1940). Histological slides were prepared by the primitive method of freehand section. Each one was sectioned in the midline, freehand sections were obtained using a razor blade, Transverse sections were subjected to double staining with safranin and fast green (Kraus and Arduin, 1997). Root sections were analyzed in the light microscope. In root section number of layers in the epidermis, endodermis, cortex and number of vascular bundles, roothairs, mycorrhiza association were observed and noted. The most important aspects were recorded with the digital camera attached to the Olympus microscope.

**RESULT AND DISCUSSION**

In this research, just the absorption roots were analyzed and are described below in sequence.

Most of the terrestrial orchids have tubers. While *Nervili*a species has tubers and also has absorption and soil fixation roots. In all roots studied the epidermis was single-layered, the cortex was relatively thickened and a number of its cellular layers varied among species. Cortex was formed by round cells of various sizes and thin walls, the cortical cell layers close to the endodermis and cells were parenchymatic. It is generally believed that the anatomy of the plant is usually less affected by environmental conditions than morphological characters (Ponert *et al.,* 2016). Therefore, they may provide valuable clues for taxonomic identifications and assessing phylogenetic relationships between taxa. The morphological and anatomical characteristics of terrestrial orchids feed constant discussions about the origin of orchids (Porembski and Barthlott, 1988) It is well known that environmental factors can influence root structure to a great extent. In terrestrial orchids, they are less stressed as they are surrounded by a medium.

***Nervilia plicata*** **(Andrews)Schtr.** Hairs were unicellular, epidermis uniseriate cells were oval-shaped, velamen cells were not observed, cortex 7-8 layers wide, parenchymatic; cells of irregular shapes and size, cells with mycorrhiza association were disposed inside cells. Endodermis uniseriate, the vascular cylinder is polyarch, six arches are present. Inconspicuous xylem and phloem strands have observed, exarch condition is present. Pith cells mostly circular thin-walled parenchymatic, oval-shaped cells. Triangular intercellular spaces were present, intercellular spaces were reduced, little storage of starch grains was observed. Xylem and phloem were easily distinguishable. Xylem elements were wide and angular.

***Nervilia infundibulifolia* BLATT.&MC CANN**

Root hairs were small, unicellular, epidermis uniseriate, cells were thick, velamen cells were not observed, cortex 10-11 layers. Cells wide and parenchymatic with rich in mycorrhiza deposition inside the cells. Intercellular spaces were a small triangular shape, the Presence of mycorrhiza is easily distinguishable. Endodermis was also uniseriate, vascular cylinder 8 arches, polyarch condition. Inconspicuous xylem and phloem strands were present and show exarch condition. Pith cells were nearly circular more or less oval to polygonal cells, thin-walled. Intercellular spaces were highly reduced. The size of the cells varied from cortex to pith. Storage of abundant starch grain is also observed. Xylem and phloem are easily distinguishable. Xylem elements were wide and

angular. Vascular elements were surrounded by sclerenchyma cells.

***Nervilia aragoana* Gaud.** In roots, hairs were unicellular, epidermis uniseriate, cells were round to oval in shape. Cortex cells were closely attached to the epidermis cell layer. Cortex 11-12 cell layers wide and are parenchymatic with mycorrhiza association, the vascular cylinder has polyarch condition with10 large arch and 2 small arches and they were intercalated strands of xylem and phloem. Pith cells mostly circular, thick-walled with small intercellular spaces. Vascular bundles have clearly visible. Xylem elements were large and angular in shape, phloem was present as a mass in between the xylem element, exarch condition is present. Mycorrhiza dispersed in the cortex region were easily distinguishable.

***Nervilia crociformis*** **(ZOLL.& MORITZI)Seidenf.**

In a cross-section of roots shows thick and unicellular root hairs, root hairs were present in a mass on the outer surface. the Epidermis is single-layered, compactly arranged cells were large, cortex layer is closely attached to the endodermis, cortex cells were 9-10 layer in thickness, cells round to oval in shape, more or less compactly arranged with less intercellular spaces, mycorrhiza was dispersed in the cortex cell surface, endodermis uniseriate, the vascular cylinder has polyarch with six large and two small arch, inconspicuous xylem and phloem strands were present and are exarch, pith cells were mostly circular and thin-walled with less intercellular spaces, storage of starch grains is observed.

*Nervilia* species were characterized by having prominent fan-shaped venation of leaves and developing flowers and leaves at different times and all above-ground parts died back at the end of the growing season(Pridgeon *et al*.2005). Anatomical information provides valuable clues for taxonomic identifications and assessing the phylogenetic relationship between taxa (Tangavelu *et.al*., 2017) In this research all the studied species have similar root structure, with parenchymatic pith and the variable number of vascular bundles i.e xylem and phloem strands. Uniseriate epidermis cells were observed, with composed of closely packed parenchymatous cells. The roots showed few bunches of root hairs on the surface and had few crests in a certain area. The cortex consisted of 7-12 layers of cells with variable thickness depending upon the thickness of the root. The cortex cells were parenchymatic and mostly circular to polygonal and isodiametric with thin walls, distinct cell shape and number of cell layers can be observed. The intercellular spaces were small and mostly triangular shaped. Abundant starch grains were observed in the reserve cells. Casparian strips were not distinguishable. The absorption root performs all the functions of support, absorption and storage of nutrients.

Many studies have shown a symbiotic relationship between fungi and orchids, According to Zots (1999), symbiotic relation is common in Orchidaceae members. According to Senthikumar and Krishnamurthy (1998), different kind of micorritics associations is there in the cortex of Orchidaceae members.

The orchids has a special kind of endomycorriza where the hyphae of fungi make characteristics pelatones inside cortical cells. The pelatones form intracellular bodies which are digested by their host cells and are important for plant nutrition (Lesica and Antibus, 1990; Senthilkumar *et al.,* 2000; Franco Pinheiro Moreira and Rosy Mary dos, 2008). In this research, mycorrhizae are observed in all studied species and they were present and distributed in cortical cells. Mycorrhizal fungi are present in orchids they are important for germination and development of the plant (Arditi,1967). The Mycorrhizal association was abundant in *N. infundibulifolia*  and *N. aragoana* then compares with the *N. plicata* , least observed in *N. crociformis*.

Vascular cylinder exhibits similar characteristics in all the studied species. Xylem and phloem were surrounding the central pith. Metaxylem faces towards the pith region, protoxylem towards the endodermis i.e exarch condition, that facilitates the conduction to the entire plant parts. 11-12 vascular cylinder was observed in *N. aragoana* six vascular cylinders was observed in *N. crociformis* eight in *N*. *infundibulifolia*. Arches depending upon the diameter of the root. Vascular elements were surrounded by a sheath of sclerenchyma fibres. Pith cells were parenchymatic round to oval shape with irregular or triangular-shaped intercellular spaces. In orchid roots, velamen is usually associated with the epiphytic habit, but also recorded in some terrestrial species (Porembski & Barthlott, 1988; Stern *et al*., 1993a; Kurzweil *et al*., 1995; Stern & Judd, 2002; Frander *et al*., 2017) and absent in some taxa (Singh, 1986; Frander *et al.,* 2017) the presence of a velamen has also been observed in other monocotyledons such as Araceae, Liliaceae, Dioscoreaceae, Amaryllidaceae, Asparagaceae, And Commelinaceae ( Dahlgreen & Clifford, 1982; Cutler et.al., 2008; Frander et al., 2017) but in case of *Nervilia* species velamen is not observed. Other anatomical characters that previously observed in roots of Orchidaceae and that were found in *Nervilia* species are the presence of single-layered epidermis, endodermis, greater thickness of the parenchyma cells in the cortex, parenchyma cells in pith with less intercellular spaces, presence of starch grains in the cortex, association of mycorrhizae fungi that help for seed germination.

Four *Nervilia* species were observed in different forest types in Shiavamogga district vegetation includes evergreen, semi-evergreen, dry deciduous forests, *N. crociformis* were also observed in scrub forest and forest openings in a district. The root of four *Nervilia* species exhibits developed anatomical features for adapting to drought conditions. Storage of starch grains, inconspicuous vascular bundle, broad xylem element, presence of abundant mycorrhiza association, these anatomical characters reflect the adaptations to their growing environment.

Results confirmed that the anatomical characters of the root in *Nervilia* species showed many xeromorphic features helps for reducing water loss and water use efficiency contribute to growing in dry terrestrial conditions, this study gives new approaches to the conservation and cultivation for these four endemic Orchid species.

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