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Original Article

Botanical Pharmacognosy of *Bacopa monnieri* (Linn.) Pennell

Madathilparambil Vasu Sudhakaran*

ABSTRACT

Backgound: *B. monnieri* (Linn.) Pennell *is a slender, creeping and mat forming herb* well adapted to grow in both terrestrial and submersed conditions. It is an important medicinal plant belongs to the family Plantaginaceae and espoused as a source for the Ayurvedic drug brahmi. **Objectives:** The present study aims to delineate the morphological and histomorpho diagnostic profile of the stem, root and leaves of brahmi and analyze their qualitative and quantitative anatomical peculiarities to support the pharmacobotanical characterizations using digital, stereo and polarized microscopic techniques. **Results:** Cross section of lamina had shown a homogeneous mesophyll fails to differentiate into palisade and spongy tissues. Lamina was amphistomatic which contained three different types of stomatal complexes *vz.*, diacytic, anisocytic and anomoteracytic. The mean number of stoma per square millimeter of leaf area was found to be higher in adaxial surface (656.9/mm^2) than abaxial (433.3/mm^2) with a corresponding stomatal index of 15.2 and 9.4 respectively. Adventitious root in cross section gave 'spokes in a wheel' appearance and shoot cortex architecture had shown honey-comb pattern of aerenchyma chambers. Vascular bundles were many, conjoint and closed, where a centra solid core of xylem encircled by phloem. Crystal ideoblasts of calcium oxalate were observed in characteristic tissues of epidermis of the leaves, mesophyll, cortical tissues of stem, and intervening walls of the file of cells of diaphragms in stem and adventitious roots. **Conclusion**: The above delineated anatomical characteristics in conjunction with aerenchyma in root and shoot tissues as an effective water tolerance mechanism to thrive prolonged submergence in water logged conditions could provide valuable tags as useful markers for pharmacological identification of the taxon.

Key Words: Plantaginaceae, Aerenchyma, Calcium oxalate crystals, Anisocytic stomata, Stomatal index.

Madathilparambil Vasu Sudhakaran*

UGC-Academic Staff College, University of Calicut, Malappuram 673 635, Kerala, INDIA.

Correspondence

Madathilparambil Vasu Sudhakaran

UGC-Academic Staff College, University of Calicut, Malappuram 673 635, Kerala, INDIA.

E-mail: dr.sudhakaranvasu@gmail.com

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INTRODUCTION

The genus Bacopa was first described by Aublet in 1775[1] and he coined the term bacopa from a Latinized form of the aboriginal name called for these plants by then indigenous caribe (American-Indian) people of the French Guiana and the type specimen for species was named as *Bacopa aquatica*.[2] In the earlier morphology-based taxonomic classification[3] the genus Bacopa was included under the family Scrophulariaceae, but the DNA- based molecular phylogenetic analyses have currently circumscribed it along with foxglove (Digitalis), hedge hyssop (Gratiola), snapdragon (Antirrhinum), and pysllium (Plantago psyllium) which were all formerly in the Figwort family (Scrophulariaceae) are now included under Plantaginaceae family.[4] The genus Bacopa comprises about 60 species of aquatic plants distributed throughout the tropical and subtropical regions of the world, most of which are native to America.[5-6] *B. monnierioides*, B. *paraguariensis*, *B. congesta*, *B. dubia*, *B. hassleriana*, *B. pedersenii*, *B.ranaria*, *B. simulans*, B. *monniera* and *B. floribunda* are some of the important species of the genus. However, *Bacopa monnieri* is the most widely distributed species amongst the genus, reported to have present in almost all the continents, and being the most diverse species with the greatest number of representatives worldwide. *B.monnieri* (Linn.) Pennell and *B. floribunda* (R.Br.) Wettstein are the two species found distributed in India.

B. monnieri (Linn.) Pennell is a small creeping amphibious herb well adapted to grow in both terrestrial and submersed aquatic conditions. It is believed that the species *B. monnieri* (Linn.) Pennell is native to India, Nepal, Sri Lanka, China, Taiwan and Vietnam and Sanskrit name for the plant 'Brahmi' often considered as evidence that it is originated from India and now widespread throughout the Asia, Africa, the Arabian Peninsula, Australia, America and the Caribbean.[7] The specific epithet monnieri was ascribed to plant in honour of French naturalist Louis-Guillaume Le Monnier (1717-1799). The plant is an important drug of the Indian material medica (*Bhavprakasha Nighantu*) and Ayurveda classical texts (the Caraka-saṃhitā, SushrutaSamhita, Yogasara-saṅgraha, Aṣṭāṅgahṛdayaṃ) and described the herb as 'brahmi'.[8-9] Brahmi is a Sanskrit word derived after Brahmā (*the creator God of the Hindu mythology*) that symbolizes creation or the creative potentials that emanate from pure awareness (or *universal consciousness*). Since the brain is the centre of consciousness, according to Ayurveda classics the herbs that bear the name brahmi are Medhyarasayana (medhya=intellect; rasayana=rejuvenating) or brain tonic that promotes subtle awareness and 'sattva' (*the pure essence of consciousness*) effective in maintaining vigour and human intellect. In Ayurveda the name

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'Brahmi' has been used to describe two distinct herbs viz., *Centella asiatica* (*family: Apiaceae)* and *Bacopa monnieri* (Linn.) Pennell. Though morpho-anatomically they are distinct plants and belonging to two separate families, but having similarity in biological actions, both these herbs are highly revered for being used as a memory enhancing, adaptogenic, anti-inflammatory, analgesic, antipyretic and antiepileptic agents in centuries old Ayurveda treatment regime. Brahmi forms an ingredient in many Ayurvedic formulations viz., Brahmirasayana (*rejuvenating medicine*), Brahmighrita (*clarified butter*), Brahmitaila (*medicated oil*), BrahmiVati (*tablet*) and Sarasvatarishta (*decoction as brain tonic*). Brahmi containing Ayurveda *formulations has been recommended as medications for the treatment of asthma, insanity, epilepsy and the management of a range of mental conditions including anxiety, lack of concentration, poor cognition and as an energizer for activating the central nervous system.*[10-11] *The herb contains a mix of synergistic compounds viz., saponins, flavonoids, alkaloids, carotenoids, amino acids, aglycans and vitamins. Because of the affluence of a host such bioactive constituents B. monnieri (BM) largely been acknowledged as a natural nootropics.* Most of the biological actions of BM have been ascribed to a group of triterpenoid saponins called Bacosides, with aglycone units of jujubogenin or pseudo-jujubogenin and alkaloids.[10,12-14]

B. monnieri has extensively been investigated by various workers for its morphological, and taxonomical, phytochemical, pharmacological, molecular, microbiological, and micropropagation studies.[15-21] However, the botanical standards specified for evaluation of intact plant seemed too scanty in literature[22-25]and available studies are not exhaustive enough to disentangle the crude raw materials from its allied/ spurious/adulterant or substitute species. Therefore, the present study was undertaken with the objectives of elaborating the morpho-anatomical characteristics of the adventitious root, stem, lamina, epidermal peel and powder microscopy of the specimens using digital, polarizing and stereo microscopy. It also aims to delineate and establish pharmacongostic markers which may provide easily recognizable tags for the regulatory perspectives of the quality control measures of the crude drug.

MATERIALS AND METHODS

Materials

The plant is a slender, prostrate, creeping and mat forming herb, which attains a height up to30-40 cm (Figure 1 & 1a). Stem is long, slender, greenish and glabrous with a tuft of long, branched adventitious roots arising from the internode at regular intervals. Adventitious roots are cylindrical, whitish cream colour. Leaf; simple, small, glossy, leaves consisted of 4-5 mm long and 0.6-0.9 mm width are borne in pairs along the stem. Lamina is sessile, thick fleshy with a prominent midvein (Figure 3a) and a few obscured lateral veins, having opposite and decussate phyllotaxy. Lamina base is ovate to lanceolate, margin entire and apex rounded. Flowers: solitary and axillary, born on a long (10 mm) pedicel, subtended by a pair of green bracteoles at the base below the calyx (Figure 2a). Sepals: 5, free, lobes are unequal, green in colour. Corolla: petals 5, companulate, 0.8mm long; faintly two lipped, lower lip 3 lobed and upper 2 lobed, pinkish white or purple in colour. Stamen: 4, didynamous, anther locules are parallel to filament (Figure 2). Stigma: capitate, green in colour, shallowly bilobed, and dilated (Figure 2). Fruit: capsule, narrowly ovoid with persistent calyx. Seeds: many, yellow-brown, usually remain hidden inside the persistent calyx.

Methods

B. monnieri was collected from its natural habitat (wet land paddy fields) from the Thiruvananthapuram District of the State of Kerala, India. The plant was identified and authenticated with the help of the Flora of Presidency of Madras. Fine hand sections of the specimens were taken using the razor blade and sections were stained with alcoholic Safranin (1%), and mounted on glass slides in glycerine. Photomicrographs of sections and powder analysis were made by using Olympus Microscope (Japan, Model CX 41) with CCD camera (2 mega pixel). Images were concomitantly viewed and specimens analysed for pharmacognostic characteristics, quantitative measurements were taken using Olympus Image-Pro Plus (version 5.1). The shade dried and pulverized powder was cleared with absolute alcohol and mounted on glass slides for powder analysis. The descriptive terms of the anatomical features were used here as per Metcalfe and Chalk, [26] Conquist[27] and Sudhakaran.[28-29]

RESULTS AND DISCUSSION

Microscopic evaluation of Leaves

Lamina is devoid of a conspicuous petiole, and appeared thick, succulent and flattened in their dorsiventral orientation with a prominent midvein running at the middle of the lamina (Figure 3 and Figure 3a). Epidermal cells of the lamina contained abundant deposition of prismatic crystals of calcium oxalate (Figure 4 and 4a) of various shapes and sizes (dimensions 9.7-17.5µm x 4.6-8.1 µm) which provide mechanical support to the delicate parenchymatous tissues. The lamina shared the general pattern of tissue organization which includes epidermis, mesophyll and vascular structures. Both adaxial and abaxial epidermises were uniseriate, composed of compactly arranged rectangular cells, enveloped outwardly by a thin cuticle. Water repellent cuticle layer avoid the wetting of leaf surfaces and help in gas exchange between stomata and outer atmosphere, especially when the plant is in submerged condition. Some of the pavement cells were modified as multiseriate glandular trichomes, in which the apical head of the trichome often appeared shrivelled or withered; leaving basal cells as leftovers (dimension; 28.2 µm x 25.4 µm), quite evident in paradermal sections (Figure 5). A homogeneous mass of 9-10 layers of isodiametic cells sandwiched between the upper and lower epidermises of the lamina to form the mesophyll. Mesophyll cells contained abundant deposition of chloroplasts (Figure 5). Another feature of the mesophyll was the uneven distribution of aeriferous gaps. Adaxial mesophyll cells were oriented tightly packed thereby leaving very small intercellular spaces at the point of their mutual contact, whereas the intercellular spaces or gaps so formed at the abaxial mesophyll were large and wide.

Epidermal characters

The epidermal peel of lamina showed three types of differential cells namely the pavement cells, kidney shaped guard cells of the stomatal complex and glandular trichomes (Figure 6). Pavement cell was the predominant type having wavy or sinuous cell wall (Figure 6, 6a, 6b and 6c) and they are pentagonal shape. Individual pavement cell occupied an average area of about 1140 µm^2. Stomata were amphistomatic and lamina contained three different types of stomatal complexes viz., as diacytic (two subsidiary cells with their conjoint walls aligned parallel to the guard cells), anisosytic (three subsidiary cells one is relatively smaller than other two) and anomoteteracytic (four subsidiary cells of which two in polar and two in lateral position on the guard cells). These stomatal distributions found to be not at random on the epidermal surface; but seemed to be aligned in a more or less diagonal fashion and stomatal orientation had followed one -celled- spacing rule (Figure 6c). Of the three different stomatal complexes, anisosytic type was found to be the predominant type (54%), followed by anomotetracytic type (29%) and the least being diacytic (17%). The mean size of the guard cell area (GCA) was found to be 458.2µm^2 for abaxial epidermis and 343.7µm^2 for adaxial.

The stomatal distribution pattern of *B. monnieri* had shown a trend akin to terrestrial dicotyledous as having the amphistomatic condition; but the number of stomata on the adaxial surface was found to be

Figure 1: Photograph of *Bacopa monnieri* (Linn.) Pennell.: showing natural habit.

Figure 1a: Photograph of *Bacopa monnieri* (Linn.) Pennell.: showing a flower.

Figure 2: *Bacopa monnieri* (Linn.) Pennell: showing a twig (stereo microscopic view x 1).

Figure 2a: *Bacopa monnieri* (Linn.) Pennell.: showing a flower (stereo microscopic view x 1).

Figure 3: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: T.S of lamina passing through midrib (x 2).

Figure 3a: *Bacopa monnieri* (Linn.) Pennell.: morphology of leaf (stereo microscopic view x 1).

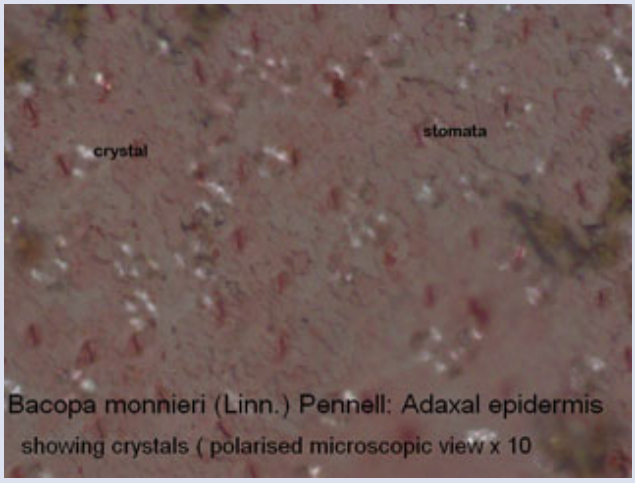

Figure 4: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: Adaxial epidermis showing crystals (polarized microscopic view x 10).

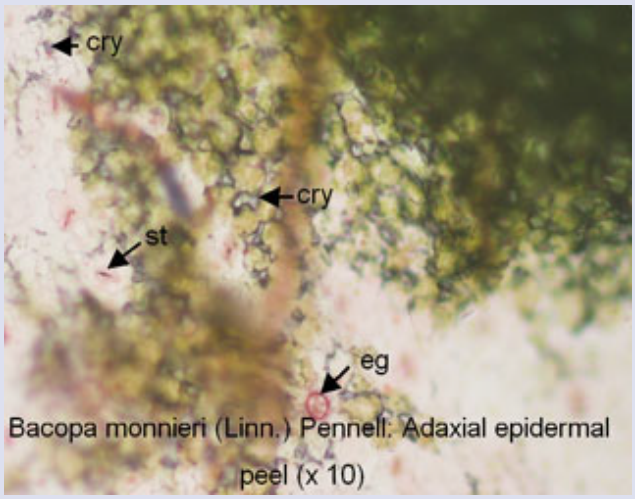

Figure 4a: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: Adaxial epidermis showing crystals (x 10).

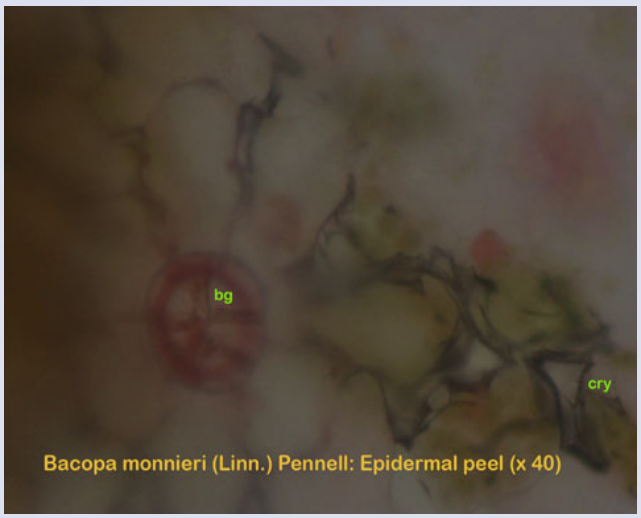

Figure 5: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: Adaxial epidermal peel showing basal cell (bg) of glandular trichome and crystals (x 40)

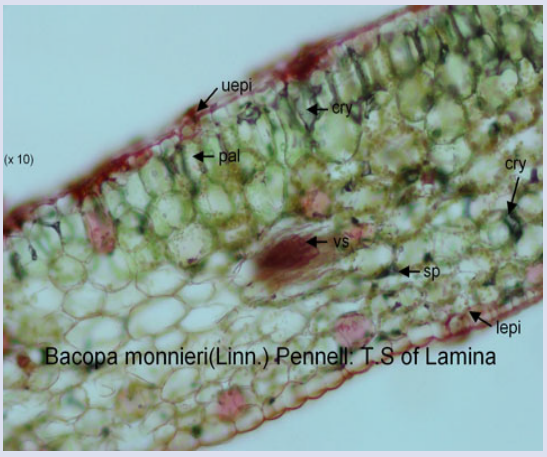

Figure 6: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: sectional view of the lamina passing through midrib (x 4)

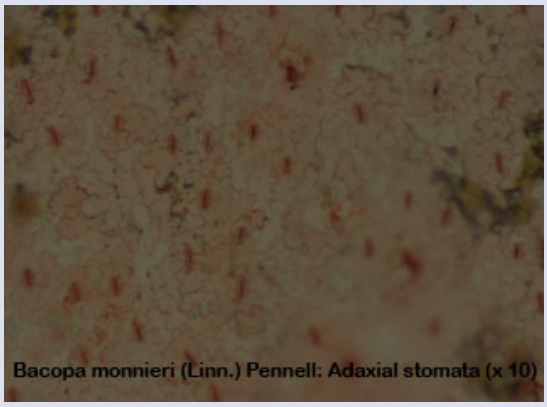

Figure 6a: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: adaxial epidermal peel showing the positioning of stomata (x 10)

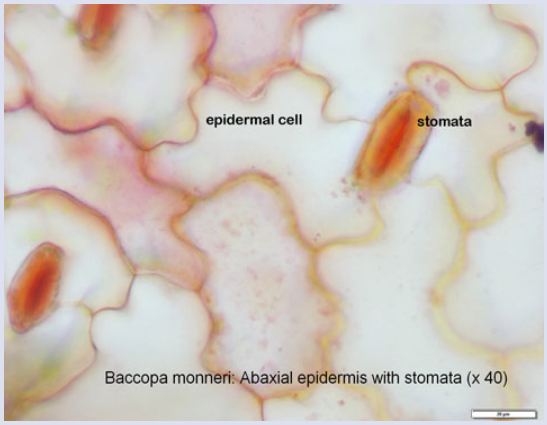

Figure 6b: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: abaxial stomata (x 40).

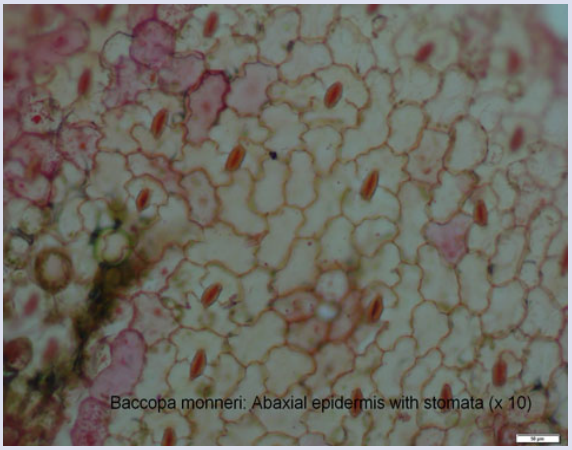

Figure 6c: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: abaxial epidermal peel showing the positioning of stomata (x 10).

Figure 8: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross section of adventitious root showing spokes in a wheel appearance (x 4).

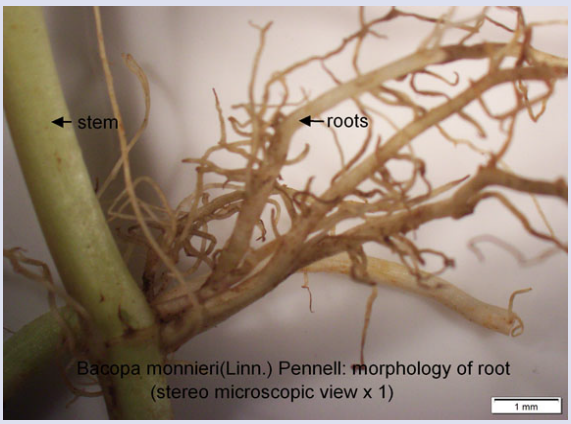

Figure 7: *Bacopa monnieri* (Linn.) Pennell.: showing morphology of adventitious roots (stereo microscopic view x 1).

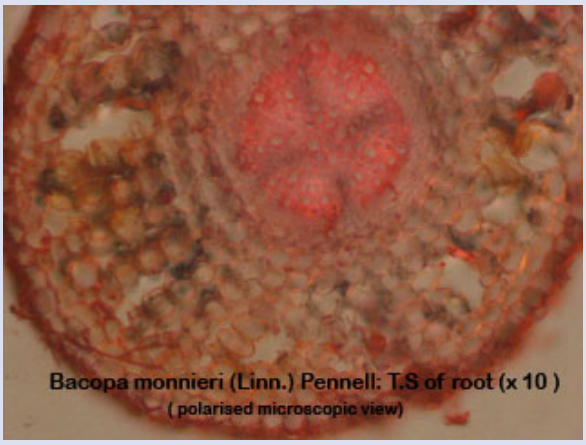

Figure 8a: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross section of adventitious root (polarized microscopic view (x 10).

Figure 7a: *Bacopa monnieri* (Linn.) Pennell.: showing apex portion of an adventitious root (stereo microscopic view x 1).

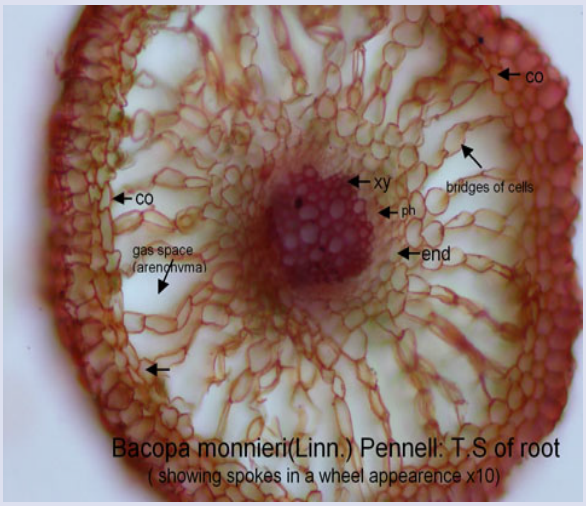

Figure 8b: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross section of adventitious root showing spokes in a wheel appearance (x 10).

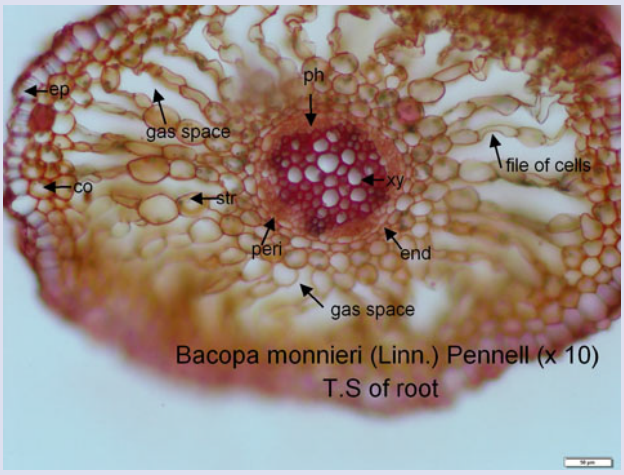

Figure 8c: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross section of adventitious root (x 10).

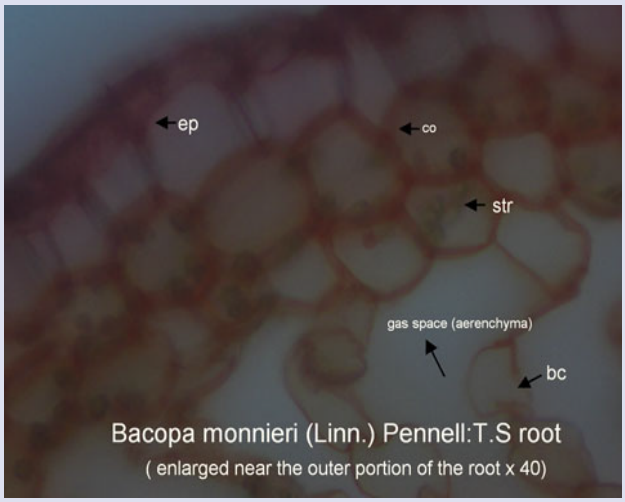

Figure 8d: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross sectional view of adventitious root, outer portion enlarged (x 40).

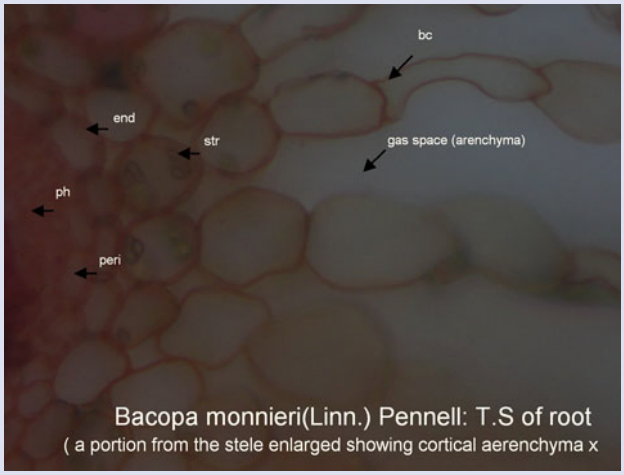

Figure 8e: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross sectional view of adventitious root, enlarged showing cortex (x 40).

more, about twice the frequency of stomata than on abaxial lamina. Such type of leaves often referred to as heterostomatic. In the present study the number of epidermal cells per square millimeter of leaf area was quantitatively estimated as 656.9 and 433.3 respectively for adaxial and abaxial sides and number of stomata per square millimeter area of lamina was estimated at 117.6/mm^2 for adaxial and 44.9/mm^2 for abaxial lamina respectively. Kaul[30] has reported a more or less similar pattern of adaxial stomatal distribution in certain wetland/aquatic species, ranged from 66/mm^2 to 120/mm^2. It was reported as 66/mm^2 for *Potamogelon coccineum*; *Ranunculus seeleratus* (72/mm^2); *Bottia cordata* (90/mm2) and for *Potamogelon nodosus* (120/mm^2). In the present study the stomatal index for B. monneri was quantitatively estimated as of 15.2 and 9.4 respectively for the upper and the lower surfaces

Microscopic evaluation of root

Bacopa monnieri roots is not originating from the seedling embryos, but a tuft of elongated, slender and branched adventitious roots aroused from the nodal regions of the prostrate stem at regular intervals (Figure 7 and 7a). Diameter of adventitious roots on an average was about 1 mm (1089µm) and appeared a circular outline in cross sections (Figure 8). Root comprised of three types of tissue system, the outer dermal layer or piliferous layer *(piliferous layer is derived from protoderm*), followed by exodermis (*also derived from protoderm*) and aerenchyma cortex (*derived from ground meristem*) and inner vascular system or stele (Figure 8 and 8a). The outer piliferous layer was uniseriate, composed of tightly oriented rectangular cells often ruptured at places interrupting the circular outline. The outer piliferous layer was subtended by a layer of exodermis. Inner to the exodermis positioned parenchymatous cortex. Cortex was very prominent which occupied about 65% of the total volume of the root. Exodermis and cortical tissues were derived from the ground meristem, however exodermis is distinct from the underlying cortical parenchyma in having subrein in their cell walls.[31] The arenchyma or gas spaces that formed in cortex seemed to be separated by bridges of cells spanning the space, which together with epidermis cells and stele giving 'spokes in a wheel' appearance (Figure 8c and 8d) to root in the cross section. This "spokes in a wheel' histo-morphology often fashioned when cortical cells undergoes radial lysogeny, where selective collapse of radial files of cells by programmed cell death and disintegration create large gas spaces or lacunae. The aerenchyma thus formed often referred to as lysigenous aerenchyma. Aerenchyma chambers formed in the root cortex were separated from one another by transversely oriented partition walls called diaphragm. A diaphragm layer comprised of about 7-8 radially elongated, rectangular cortical cells. Such a radial files of cells forming the diaphragm and exodermal cells they often contained simple and compound starch grains (Figure 8e and 8f). Endodermis was quite distinct in cross section as layer of cells which demarcating the inner stelar region from the outer cortex (Figure 8g). Subtending the endodermis formed 1-2 layered pericycle, which encircled the vascular system. Vascular system (stele) constituted to form about 30% total volume of the root, and vascular strand appeared conjoint and closed type, where a solid core of xylem at the center encircled by phloem (Figure 8g). Vessels were quite few in number, larger and smaller vessels were seemed to be aligned intermittently. Virtually there exists no cortical sclerenchyma tissues in root, however the prismatic calcium oxalate crystals embedded in the intervening wall of files of cells in the diaphragm and crystals idioblasts associated with arenchyma tissues could confer mechanical strength to the delicate parenchymatous cortex. Moreover, crystal idioblasts associated with cortical cells sometimes promote the formation of air space while the plants in submerged condition. Where degradation of calcium oxalate crystals by Oxalate oxidase (OXO) enzyme (oxalate:oxygen oxido reductase, EC1.2.3.4) could produce

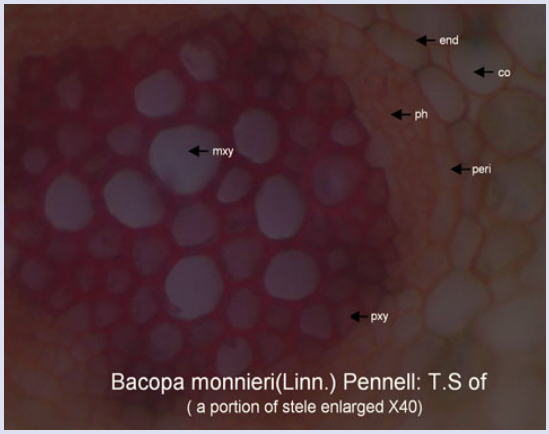

Figure 8f: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross sectional view of adventitious root, a portion of stele enlarged (x 40).

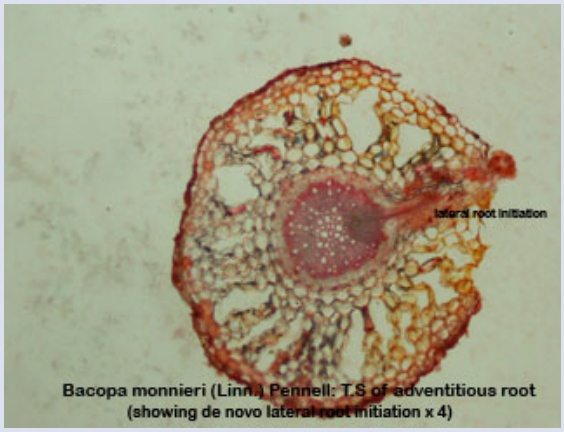

Figure 8g: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross sectional view of adventitious root, showing de novo lateral root initiation (x 4).

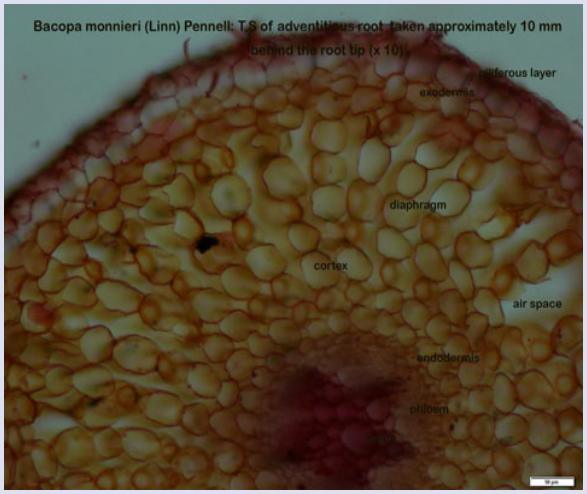

Figure 9: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross sectional view of adventitious root taken from 10 mm behind the apex of root (x 10).

Figure 9a: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross sectional view of adventitious root taken from 10 mm behind the apex of root- middle portion enlarged (x 10).

high concentration of hydroperoxide (H_2O_2) which in turn trigger cell lysis and disassociate the crystal idioblasts, forming new arenchyma gas spaces have been reported in some aquatics.[32-34]

Drew *et al.*[35] have opined that aerenchyma formation is the most effective water logging tolerance mechanism in wetland species as it provides buoyancy and low gravitational force to prolonged submergence. Cell destruction causing internal gas spaces not only increases the tissue porosity; but interconnected pathways of gas spaces so formed facilitate the long distance transport of atmospheric O_2 via the stomata to aerial shoot and finally to tip of the root. Further it helps in the removal of noxious gases such as methane, Co_2 and ethylene. At the same time air space formation also simultaneously reduces the number of living cells (biomass) that consume oxygen. Thus aerenchyma formation is an essential adaptive feature for aquatic/wet land life.

Lateral root initiation from parental adventitious root

de novo lateral root initiation in *B. monneri* seemed to be occurred from the pericycle (Figure 8g) specifically from a few or a limited number of pericycle cells at the xylem pole of parent (adventitious) root. During lateral root emergences wall loosening happen in the overlying tissues of endodermis, cortex and epidermis of the of parental adventitious root and the emerging root primodia pushes the overlying layers of these cells in order to come out from the parent root (**Figure 8g**) and penetrate into the soil environment.

Adventitious roots at a distance of one centimeter behind the root tip

Degree of collapse and disintegration of cortical rows or files of cells were much pronounced as (adventitious) root getting old and matured. Cross sections taken from the root tips (Figure 9 and 9a) were compared with those taken from root near to nodal region, and a distance of one centimetre behind the root tip. The result showed that root tip cross sections had shown a characteristic increase in number of diaphragm layers in the cortex. This may be due to spatially selective death of grown cells increases as the adventitious root grows or in other words, the progress of aerenchyma formation is dependent on the age of the tissue.

Microscopic evaluation of stem

Stem is grabrous, cylindical, and green (Figure 11), having an average cross sectional diameter of about 1.5 mm (1562.1µm). T.S of the stem appeared circular in outline which consisted of an outer epidermis, followed by arenchymatous cortex, endodermis, vascular strands and parenchymatous pith at its center (Figure 11). Cortex formed the major portion of the tissues of stem, which constituted about 60% of total volume of the stem (Figure 11a and 11b). Epidermis was uniseriate, consisted of tightly aligned rectangular cells which enveloped outwardly by thin cuticle. Towards inside the epidermal layer formed a layer of cortical cells called exodermis, having their walls modified by deposition of suberin. Exodermis act as a hydrophobic barrier (suberin) preventing the free diffusion of molecules between the inner cortical parenchyma and roots outer environment.[31] Underneath the exodermis contained several layers (2-10) of cortical cells. Cortical cells were of different sizes, they include round, pentagonal, hexagonal and polygonal cells (Figure 11c and 11d). There were a group of 8-10 cells in peculiar geometric fashion aligned to form the partition walls or diaphragm (Figure 11c and 11d), which completely encircle the gas spaces (aerenchyma). Files of cells of the adjacent diaphragm layers were jointed one another and interlocked. This pattern of development of cortical aerenchyma is often referred to as honey-comb like pattern.

There are mainly two types of arenchyma formations in primary cortical tissues have been illustrated in literature namely lysigenous and schizogenous by Evans.[36] Their mechanism of development and differentiation are quite different; however, they could easily be identified histo-morphologically by the presence of the remnants of cell wall materials according to Smirnoff and Crawford.[37]

The patterns of alignment of diaphragm layers and interlocking of adjacent diaphragms impart definite shape and contour to gas spaces (as of tetragonal, pentagonal or hexagonal shape) of B.monnieri. The number cells that constituted an individual diaphragm layer in *B.monneri* were found to be either 8 or 10. Adjacent diaphragm layers so formed were physically interlocked to one another by sharing their cells to form an interconnected mesh-like configuration in the shoot cortex as honey-comb pattern. The gas spaces were of different sizes and the surface area occupied by individual cortical gas space ranged from 1020µm^2 to 7022.3µm^2. The Arenchyma gas spaces that occupied in the middle of the cortex were of large sized (Figure 11f) and often seemed to have covered an area of 6920 µm^2 to 6920.5µm^2. Cortical parenchyma appeared to have contained crystal idioblasts coupled with arenchyma chambers (Figure 11h). Some of bridge cells that formed the diaphragm found to have contained transversely oriented

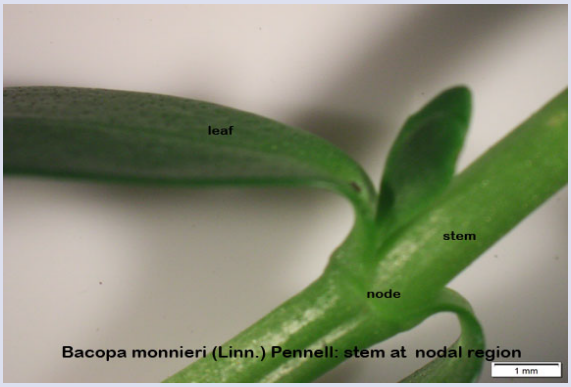

Figure 10: *Bacopa monnieri* (Linn.) Pennell.: morphology of stem at nodal region (stereo microscopic view x 1).

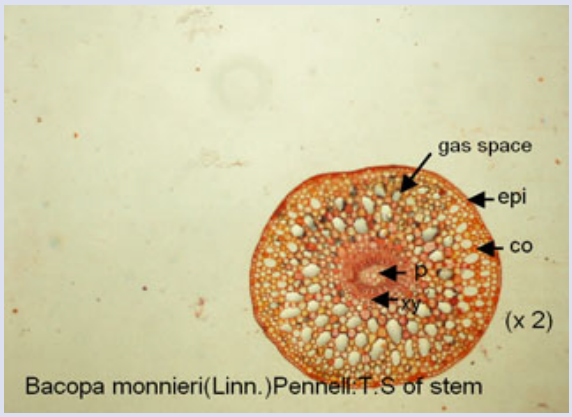

Figure 11: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross section of stem (x 2).

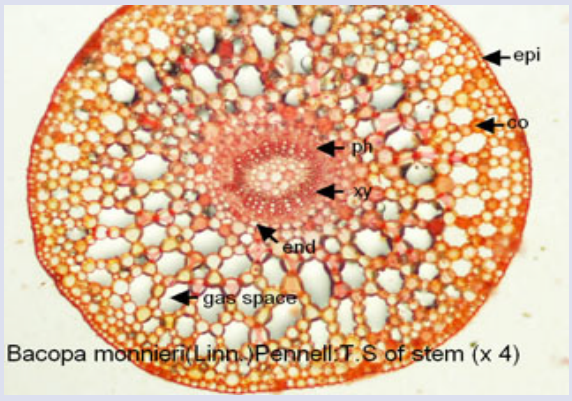

Figure 11a: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross section of stem (x 4).

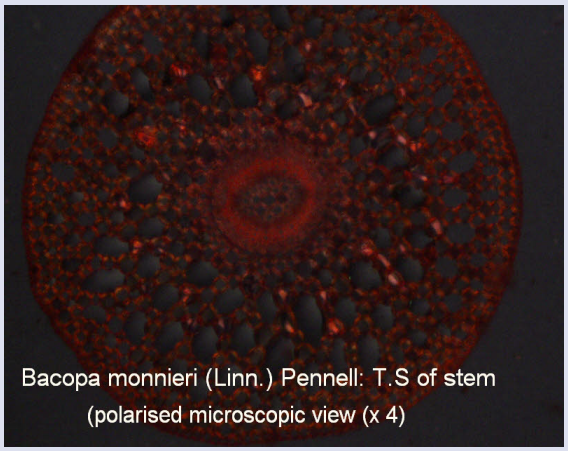

Figure 11b: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross section of stem (polarized microscopic view x 4).

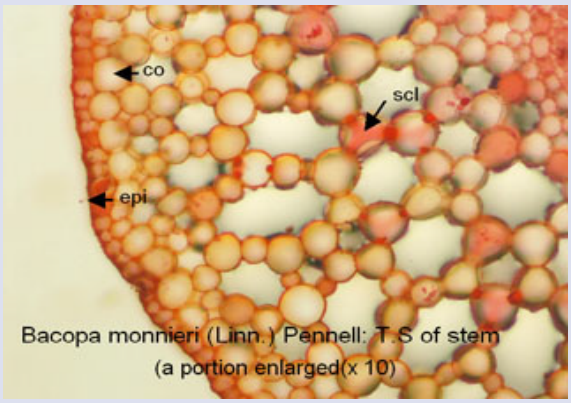

Figure 11c: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross sectional view of stem; outer portion enlarged (x 10).

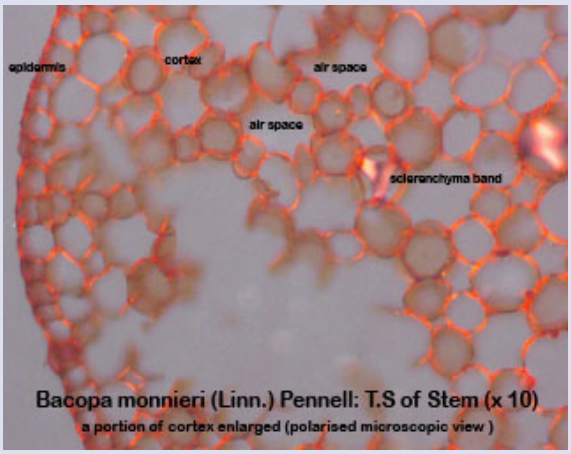

Figure 11d: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross sectional view of stem; middle portion enlarged (polarized microscopic view x 10).

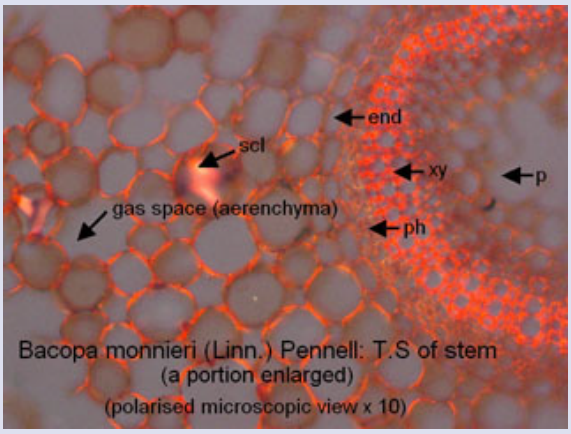

Figure 11e: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross sectional view of stem; a portion of steel enlarged (polarized microscopic view x 10).

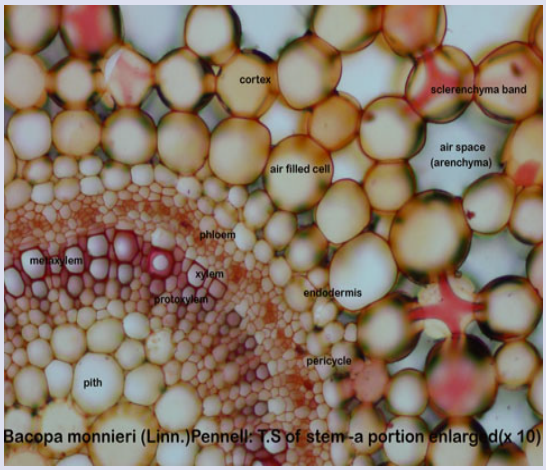

Figure 11f: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross sectional view of stem; a portion of steel enlarged (x 10).

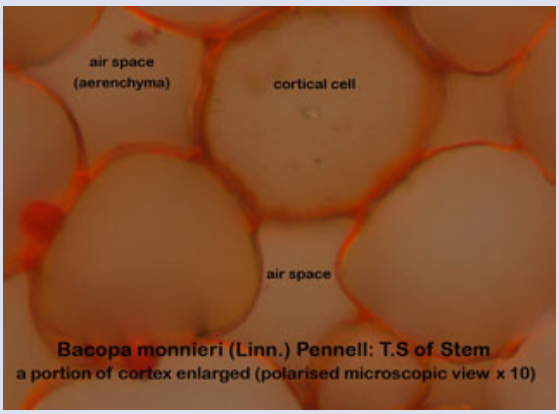

Figure 11g: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross sectional view of stem; a portion of cortex enlarged (polarized microscopic view x 10).

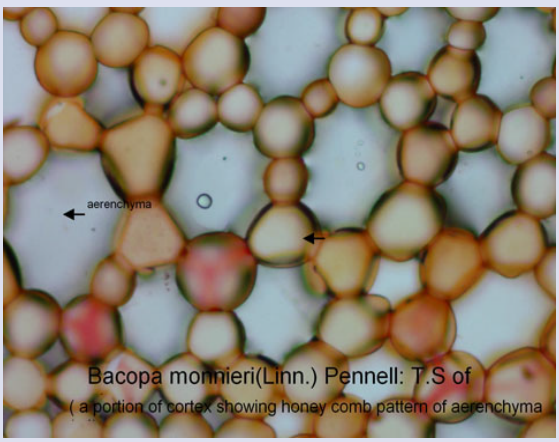

Figure 11h: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross sectional view of stem; a portion of the middle of cortex enlarged (x 10).

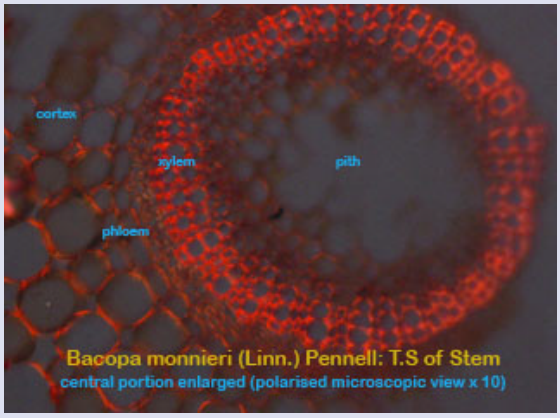

Figure 11i: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross sectional view of stem; central portion enlarged (polarized microscopic view x 10).

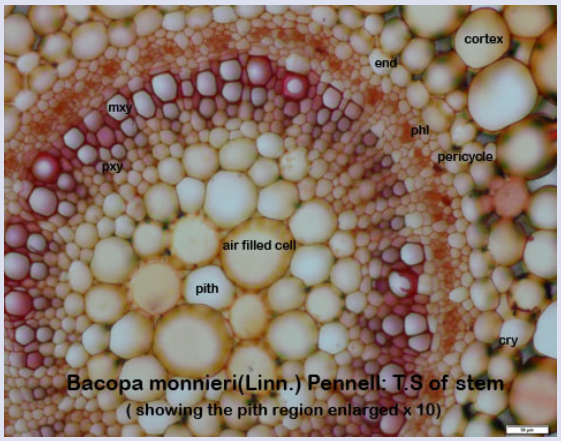

Figure 11j: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross sectional view of stem; central portion enlarged (x 10).

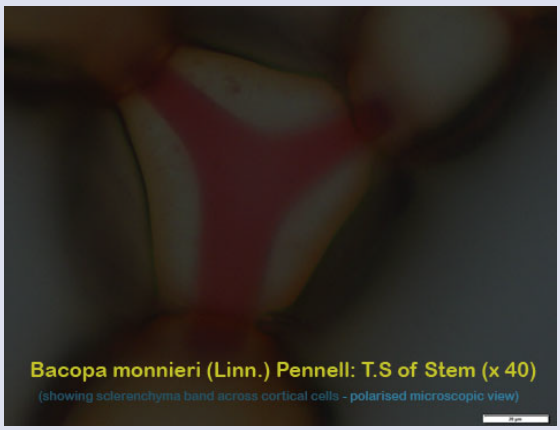

Figure 11k: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross sectional view of stem; showing sclerenchyma band across cortical tissues (polarized microscopic view x 40).

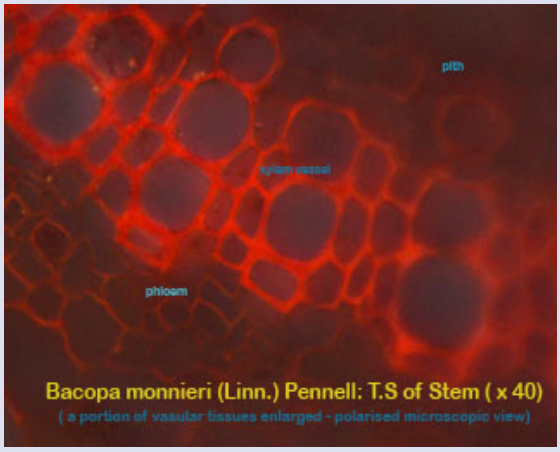

Figure 11l: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: cross sectional view of stem; a portion of vascular tissues enlarged (polarized microscopic view x 40).

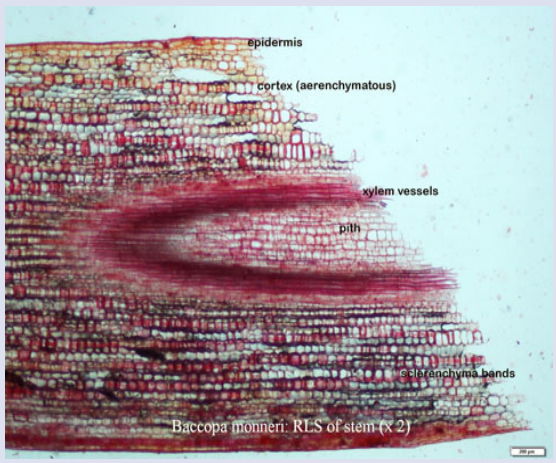

Figure 12: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: Radial longitudinal section of stem (x 2).

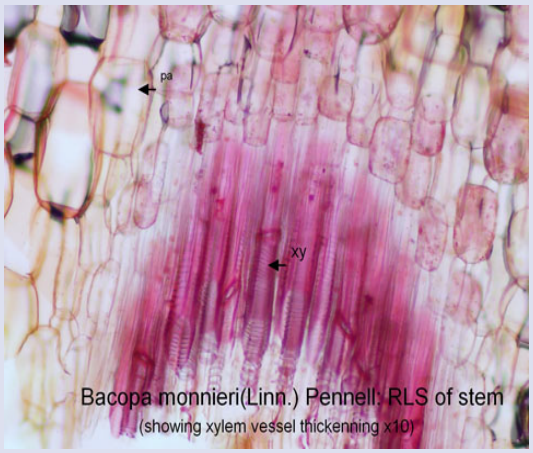

Figure 12a: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: Radial longitudinal section of stem; showing vessels thickening (x 10).

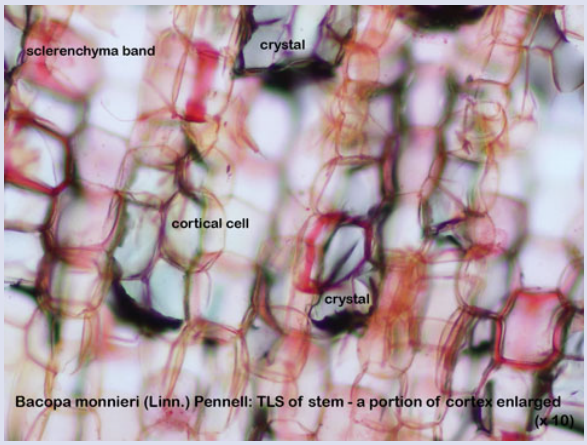

Figure 12b: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: Transverse longitudinal section of stem; a portion of cortex enlarged showing crystals (x 40).

Figure 12c: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: Radial longitudinal section of stem; showing vessels thickening (x 40).

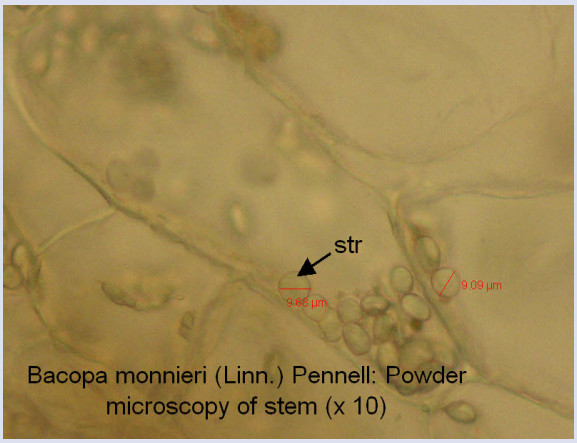

Figure 13: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: Powder microscopy of stem; showing starch grains (x 40).

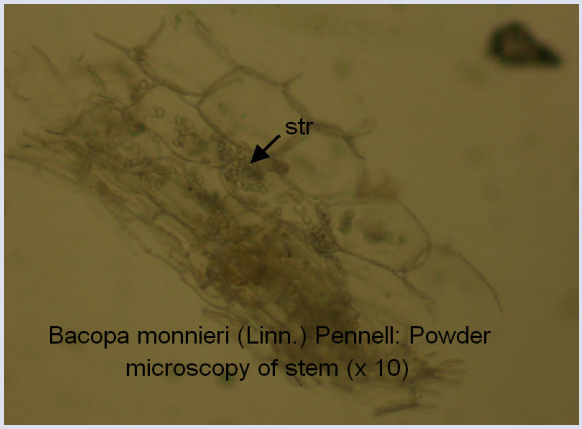

Figure 13a: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: Powder microscopy of stem; showing starch grains (x 10).

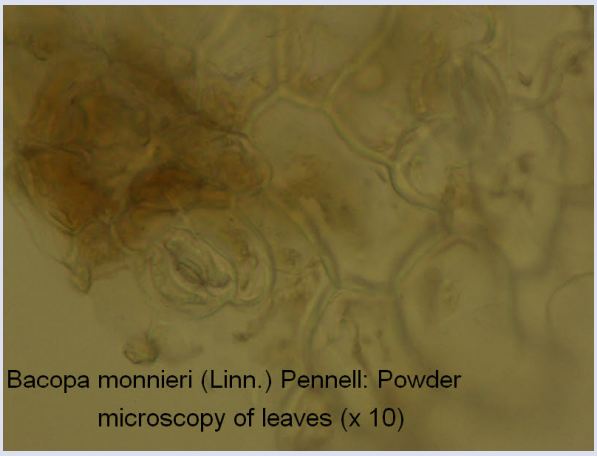

Figure 13b: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: Powder microscopy of stem; showing cortical parenchyma cell (x 10).

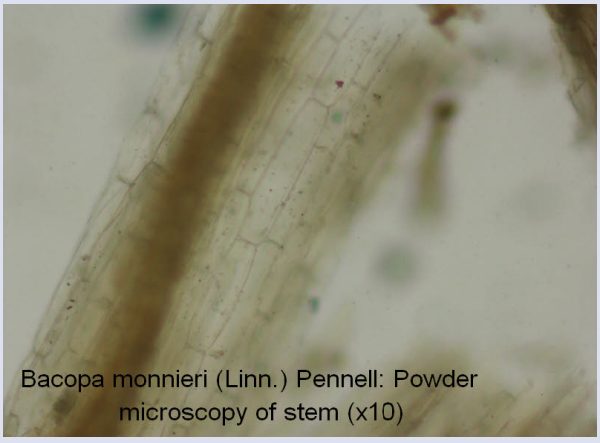

Figure 13c: Photomicrograph of *Bacopa monnieri* (Linn.) Pennell.: Powder microscopy of stem; showing cortical parenchyma cell (x 40).

strip of sclerenchyma or sclerenchyma bands (Figure 11e, 11f, 11g). In addition to prismatic crystals, the sclerenchyma bands that traverse across the diaphragm layers also could confer mechanical strength and stiffness to delicate cortical parenchyma. A conspicuous endodermis was distinct (Figure 111, 11j) in the cross section of stem. Underlying to the endodermis formed pericycle. Vascular bundles were many, conjoint and closed. Xylem and phloem were distinct and separate. The thickened endodermal cells that surround the vasculature could help in preventing water ingress into the aerenchyma from the osmotically pressurized xylem elements of the stem[38] when the plant in submerged condition.

Tangential longitudinal sections of stem

The primary xylem tissue was differentiated into metaxylem and proxylem. Two distinct secondary wall lignification patterns were observed in vessel elements viz., spiral thickening and pitted thickening (Figure 12a, 12c). Whole inner wall of the metaxylem was uniformly reinforced with secondary thickening of lignin (pitted thickenings), leaving small pits area, while in protoxylem polymerization was restricted in the form of rings at the inner surfaces of cell wall as spiral bands (Figure 12a, 12c). The number of vessels in shoot was not many in *B. monneri* and vessels appeared aligned in radial row of 3-4. Vessels were polygonal in shape and mean of vessel diameter was found to be of 33 µm. Inter vessel pitting were found to be in opposite position on radial walls and biseriate stagged pits having a mean pit aperture of about 3.7µm. Parenchymatous porous pith occupied at the centre, pith cells were inflated with filled air and cells appeared polygonal in shape. Air filled 2-3 cells that occupied at centre were found to be remarkably large in size. Cortical parenchyma of the pith, particularly cells that lining a few inner layers and their intervening walls found to have embedded with prismatic crystals of calcium oxalate (Figure 12b).

Powder microscopy

The dried leaves, stem and root of *B. monnerie* were analyzed for powder characteristics. Microscopic examination showed fragments of leaf epidermis with conspicuous stomata (Figure 13a 13b).Stem powder showed fragments of elongated rectangular pharenchyma cells (Figure 13c) having deposition of starch grains (Figure 13) and individual starch grain appeared oval or oblong in shape and measured a mean diameter of 9.09µm.

CONCLUSION

The results of present study showed the occurrence of crystal idioblasts containing calcium oxalate of diverse forms and sizes in epidermis of the leaves, mesophyll, cortical tissues of stem, and intervening walls of the file of cells of diaphragms of adventitious roots. Since aquatic and wetland angiosperms species generally have low proportion of strengthening tissues; because of the fact that sclerenchyma formation a metabolically energetically costly affairs to them, where, crystal idioblasts in characteristic organs could confer mechanical support to the delicate parenchyma tissues. Thickened cells of endodermis that surrounding the vasculature of the stem may help in preventing water ingress into aerenchyma from osmotically (turged) pressurized xylem elements of the stem when plant exposed to water logged condition. Thus the gas-filled aerenchyma chambers act as aerating ventilator (buoyancy) and reservoir of oxygen rather than fluid filled have adaptive values in wetland habitat.

Similarly, anatomical features of reduction in lignified tissues in characteristic organs and xylem elements represented by vessels alone in lamina and adventitious roots could explain why Bacopa species-although not strictly terrestrial is found growing amphibious or swampy habitats. According to Striker *et al.*[39] though wetland species are poor in mechanical tissue proportions, an ordered pattern of alignment of multiseriate rings of parenchyma cells in the cortex would be able to maintain the mechanical strength to the plant body.

CONSENT

Not applicable.

CONFLICTS OF INTEREST

Author has declared that no conflict of interest exists.

ABBREVAITIONS

BC: bridge cell; CK:Cork; CO:Cortex; CRL, CRY: Crystal; EG, BG: basal cell of glandular trichome; END: endodermis; EPI: Epidermis; GT: Ground tissues; LEP: Lower epidermis; MXY: metaxylem; PAL: palisade; PERI: pericycle; P, PH: Phloem; V: Vessel; VB: Vascular bundle; SCL:Sclereids; SPO: spongy mesophyll; ST, STR: Starch grain; S.XY:Secondary xylem; XY:Xylem; UEPI: Upper epidermis.

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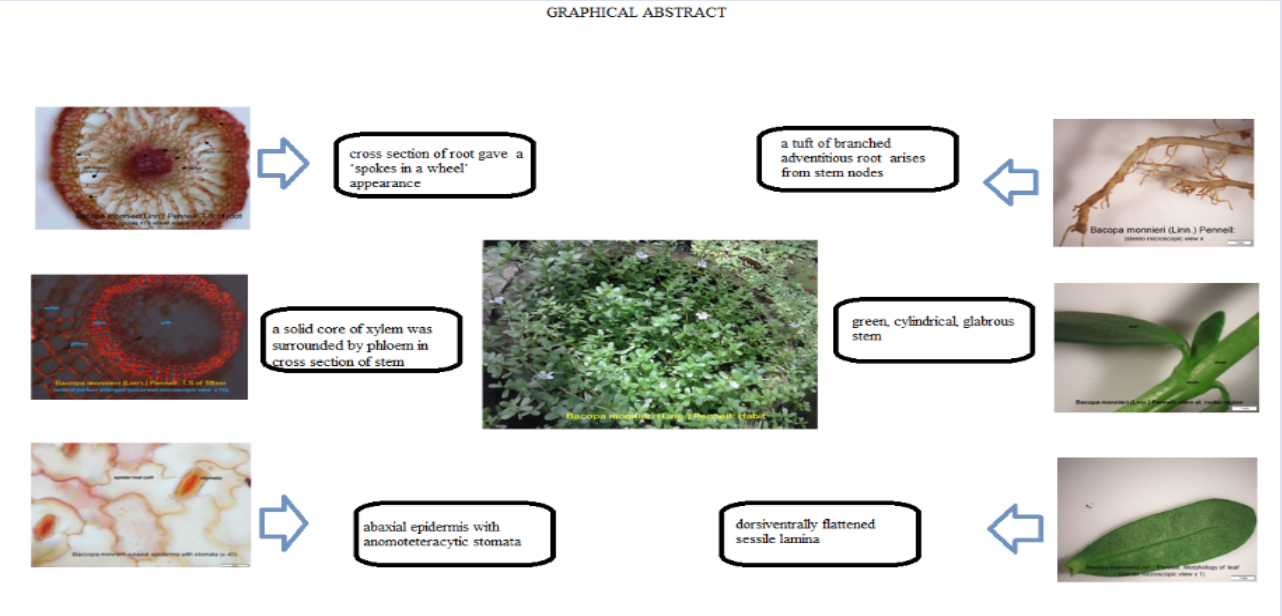
GRAPHICAL ABSTRACT



SUMMARY

- B. monnieri (Linn.) Pennell *is a slender, creeping and mat forming amphibious herb* well adapted to grow in both terrestrial and submersed aquatic conditions.
- Adventitious root in cross section gave 'spokes in a wheel' appearance with distinctive geometry and contour of aerenchyma chambers.
- Shoot cortex architecture had shown a regular mesh work of hexagonal or pentagonal or tetragonal gas spaces organized into honey-comb pattern.
- Lamina had shown amphistomatic condition with three types of stomatal complexes viz., diacytic, anisosytic and anomoteteracytic stomata.

ABOUT AUTHORS

Madathilparambil Vasu Sudhakaran (M.V.Sudhakaran): Has received the Ph.D Degree of the University of Kerala. Currently he is positioned as Associate Professor, UGC-Academic Staff College of the University of Calicut. He is a research guide in Botany under the faculty of Science. His research interests include genetic diversity analysis, genetic effects of inbreeding, anti-aging potentials of AMLA (Emblica officinalis Gaertn.), pharmacognostical and phytochemical aspects of medicinal plants.

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