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HISTOLOGICAL AND MORPHOLOGICAL RESPONSES OF *BACOPA MONNIERI* (L.) PENNELL IN GLYCOPHYTIC AND HALOPHYTIC CONDITIONS

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ABSTRACT: *Bacopa monnieri* (L.) Pennell (family Plantaginaceae) is an important therapeutic glycophytic plant used in the treatment of various ailments. It is found to grow even in brackish conditions. While surviving in such conditions, the plant evolves various adaptive mechanisms. The present study investigates the histological and morphological responses of *Bacopa monnieri* grown hydroponically under glycophytic and halophytic conditions. Plants were grown in Hoagland solution and subjected to various salt (NaCl) treatments. Plants cultivated without salt stress served as the control. Morphological analysis was done with the help of the graduated scale and graph paper. Histological investigations were carried out by hand sectioning followed by safranin and glycerine treatment. Photographs were taken with the help of Leica M 80 Stereomicroscope and Mag Cam. Significant morphological changes were observed regarding the leaf area, root and shoot length, tolerance index and stomatal index. The histological analysis revealed the presence of salt glands, starch granules, thickened cuticle, aerenchymatous cortex, prismatic crystals of calcium oxalate, phi shaped thickenings and Y, X shaped cytoplasmic strands in response to salt stress. The histological and morphological changes are the result of the mechanisms adopted by the plant to cope up with the saline stressful conditions.

INTRODUCTION: Environmental abiotic stresses such as salinity, drought, high temperature, and irradiance severely affect plant growth and production. Among all, soil salinity represents one of the most important environmental stresses, which causes serious threats to agriculture and also results in the deterioration of environment [1]. During the dry season, salts tend to accumulate in the soil because of high evaporative demand and insufficient leaching of ions due to low precipitation [2].

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Therefore, soil salinity becomes a key factor in determining crop production and distribution in many areas. The problem of salinization is regularly increasing due to irrigation with low-quality water and improper agricultural practices. Irrigated land is especially at risk with approx one-third being affected significantly by salinity. Soil salinity alone is becoming widespread in many regions and may result in serious salinization of more than 50% of all arable lands by the year 2050. With the need to irrigate more and more land to provide food for the growing human population, the design of high yielding crops with increased resistance to salt stress is becoming an important global issue [1, 3, 4]. Plants are either termed as glycophytes or halophytes by their salt tolerance capabilities.

Glycophytes are salt-sensitive plants whereas halophytes can survive and complete their life cycle in salt concentrations [5]. Salinity induces a wide range of perturbations at the cell and whole plant levels. Mangroves possess several characteristics such as salt secretion, viviparous germination, ultra filtration [6], ion sequestration and regulation of various gene expressions [4] which make them functionally and structurally adapted to saline conditions. Processes such as seed germination, seedling growth, vigor, vegetative growth, flowering and fruit set are adversely affected by high salt concentration, ultimately causing diminished economic yield and also the quality of products. Salinity stress results in increased water uptake and electrolyte leakage [7]. It results in reduced root and shoots length, germination percent and dry weight content [8]. It also causes a drastic decline in chlorophyll and carotenoid content [9]. The consequence of all these can ultimately lead to plant death as a result of growth arrest and molecular damage. To achieve salt-tolerance, the foremost task is either to prevent or alleviate the damage or to re-establish homeostatic conditions in the new stressful environment [10].

Salinity induces a wide range of perturbations at the cell and whole plant levels. Salt stress results from some detrimental processes including the toxic action of Na^+ and Cl^- ions, the impairment of mineral nutrition, the modification in the water status of the plant tissues and secondary stresses such as oxidative stress linked to the production of toxic reactive oxygen intermediates. Generally, plants exposed to salt stress change a multitude of metabolic processes. So the ability of plants to counteract the damaging effects of salt stress can be judged by examining various physiological and biochemical attributes such as water relations, inorganic nutrients, compatible solutes, hormonal regulation as well as oxidative defense metabolism [11].

Even though the effect of salinity stress has been investigated in some plants, effect of these stress on plants in general as well as medicinally/ economically important plants in particular , have not yet been elucidated. Similarly, hydroponic investigations on histological adaptations evolved due to salinity stress are very scanty. The present study investigates the morphological and histological adaptations of *Bacopa monnieri* grown hydroponically under glycophytic and halophytic conditions.

MATERIALS AND METHODS:

Plant Material: Plant cuttings were collected from the coastal regions of Northern Kerala, washed with water and then grown in Hoagland solution for cultivation. Modified Hoagland solution (Epstein [9], 1972) prepared as described by [10] Taiz and Zeiger (2002) was used for the hydroponic study. Good quality glass bottles of size 8.5 cm height and 5.5 cm diameter were used for the setting of a hydroponics system. Growth performances were observed, and most profusely rooted plants were selected for treatment with various concentrations of salt (NaCl) solutions. Continuous propagation of plants was done throughout experimentation.

Treatment with NaCl: Screening tests was done by subjecting the propagules to various NaCl treatments (0 mM, 10 mM, 20 mM, 30 mM, 40 mM, and 50 mM) and the concentration in which the propagules survived but exhibited approximately 50% growth retardation was selected as the treatment. Plants cultivated in Hoagland solution without any salt stress served as the control.

Sampling: Samples of treatments and control were collected at a comparable interval of five days up to 25 days of growth. At each interval, plants were harvested and subjected to morphological and histochemical analysis.

Morphological Analysis: Growth of plants was assessed regarding root/shoot length, leaf area, tolerance index, and stomatal index.

Root and Stem Length: The sampled propagules were washed in distilled water, blotted, and root/stem length and leaf area were measured manually, using a graduated scale and graph paper. Measurements of not less than five propagules were recorded each time.

Tolerance Index Percentage: Tolerance Index percentage was calculated according to the method of [11] Turner (1994).

TI = Observed value of root length in solution with treatment × 100 / Observed value of root length in solution without treatment

Stomatal Index: Stomatal density on abaxial and adaxial sides of the leaf was counted under a light microscope, by using nail polish impressions of leaf surface. Stomatal index was calculated according to the method of [12] Meidner and Mansfield (1968).

Stomatal Index = Number of stomata per unit area × 100 / Number of stomata + number of epidermal cells per unit area

Anatomical Analysis: Anatomical investigations were carried out by hand sectioning followed by safranin and glycerine treatment before observation. Photographs were taken with the help of Leica M 80 Stereomicroscope and Mag Cam.

RESULTS AND DISCUSSION: *Bacopa monnieri* exhibited significant morphological changes concerning leaf area, leaf number, root/shoot length, tolerance index, and stomatal index when subjected to NaCl treatments (0 mM, 10 mM, 20 mM, 30 mM, 40 mM and 50 mM). Based on the preliminary screening experiments, 50 mM NaCl was selected as the standard concentration for further analysis.

Morphological Analysis:

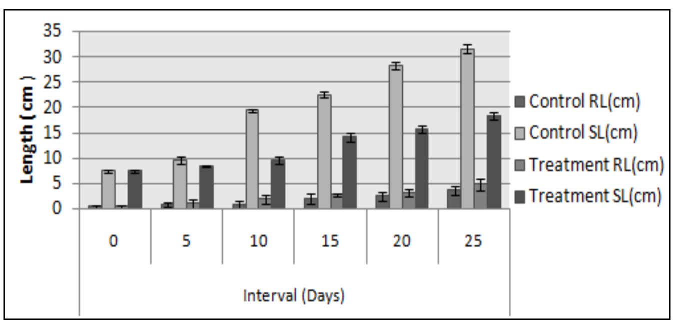
Root Growth: Growth retardation expressed regarding reduced root/ stem length and leaf area was observed in *Bacopa monnieri* as a result of Sodium chloride treatment. Due to the salt treatment, root growth was reduced fourth day onwards gradually, and the same trend continued and on 20th day 50% growth reduction was observed when compared to the control **Graph I**.

Stem Growth: Stem growth of *Bacopa monnieri* was affected by Sodium chloride treatment resulting in significant reduction of stem length in all stages of growth compared to the control **Graph 1**.

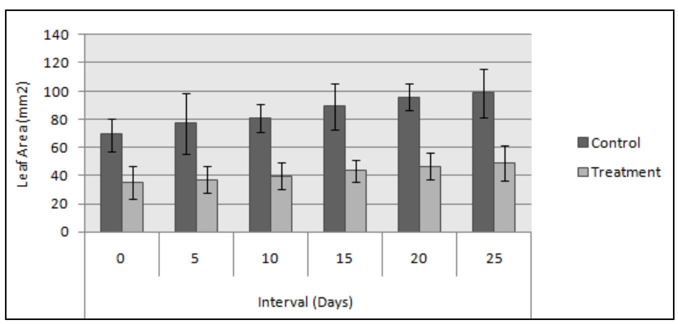
Leaf Area: Leaf growth was found to be significantly reduced due to the treatment of various concentrations of Sodium chloride. About 50% leaf growth retardation was shown by the treatments **Graph 2**.

Tolerance Index: Values of tolerance index evaluated by relative root length of experimental plants and control showed that maximum values were shown in the first interval in all the three treatments of the plant tissues **Graph 3**.

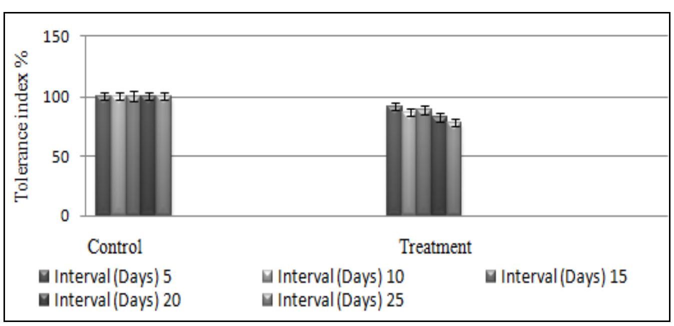
Stomatal Index: *Bacopa monnieri* treated with different concentration of sodium chloride showed a significant increase of stomatal index in the lower epidermis in comparison with that of control, whereas the stomatal index of upper epidermis remained almost unchanged **Graph 4**.



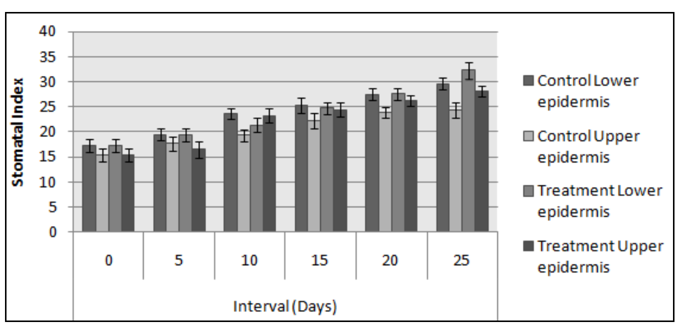
GRAPH 1: EFFECT OF NaCl ON ROOT AND SHOOT LENGTH OF *BACOPA MONNIERI*



GRAPH 2: EFFECT OF NaCl ON THE LEAF AREA OF *BACOPA MONNIERI*



GRAPH 3: EFFECT OF NaCl ON TOLERANCE INDEX % PERTAINING TO ROOT LENGTH OF *BACOPA MONNIERI*



GRAPH 4: EFFECT OF NaCl ON STOMATAL INDEX OF LEAF TISSUES OF *BACOPA MONNIERI*

Histological Analysis: The histological analysis of stem, root, and leaf (control and treatment) of *Bacopa monnieri* revealed significant adaptive modifications developed by the plant to withstand salinity stress **Fig. 1** and **2**.

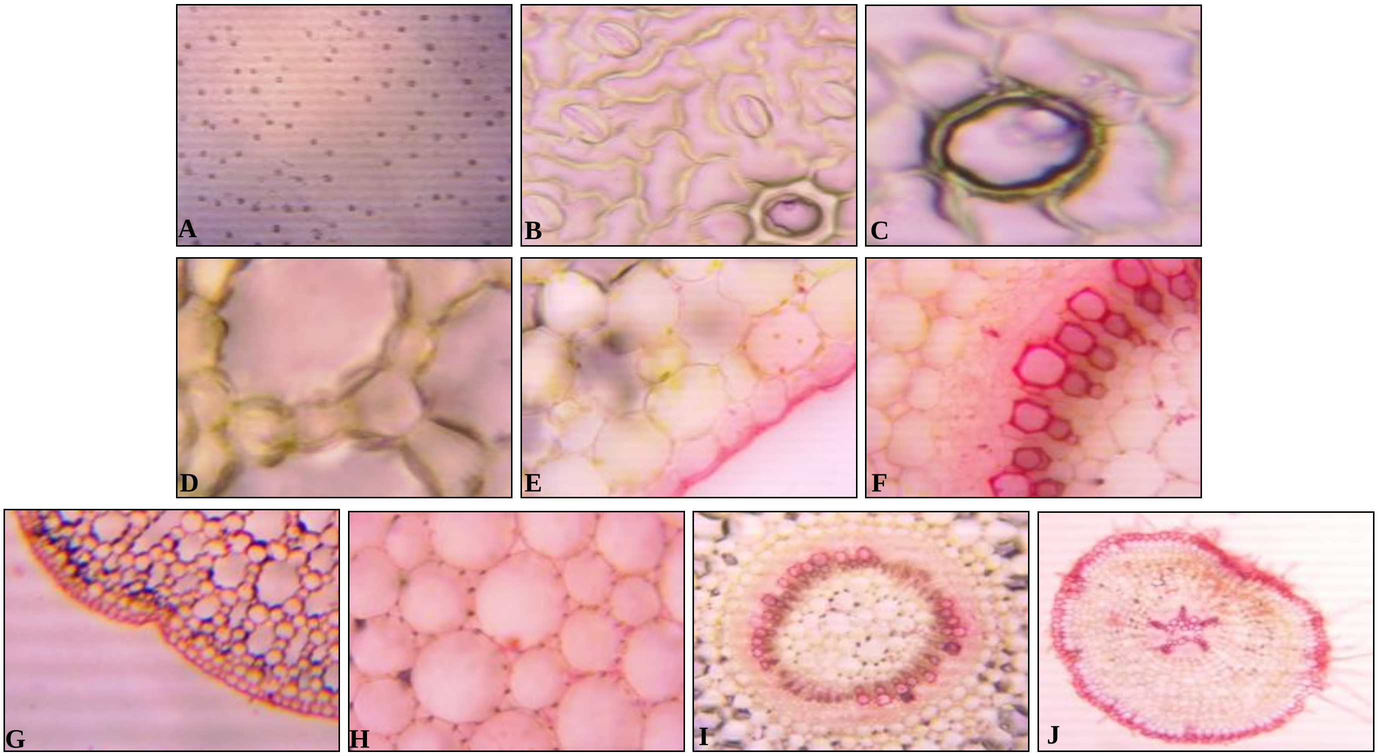


FIG. 1: *BACOPA MONNIERI* (CONTROL)- A] SURFACE IMPRESSION OF LEAF SHOWING SALT GLANDS, B] STOMATA, C] ENLARGED VIEW OF SALT GLAND, D] AERENCHYMA, E] EPIDERMAL REGION WITH CUTICLE, F] XYLEM ELEMENTS, G] CORTICAL CELLS OF STEM, H] PRISMATIC CRYSTALS OF CALCIUM OXALATE, I] WIDE LAYER OF PHLOEM, J] SECTION OF ROOT SHOWING ROOT HAIRS AND VASCULAR BUNDLES

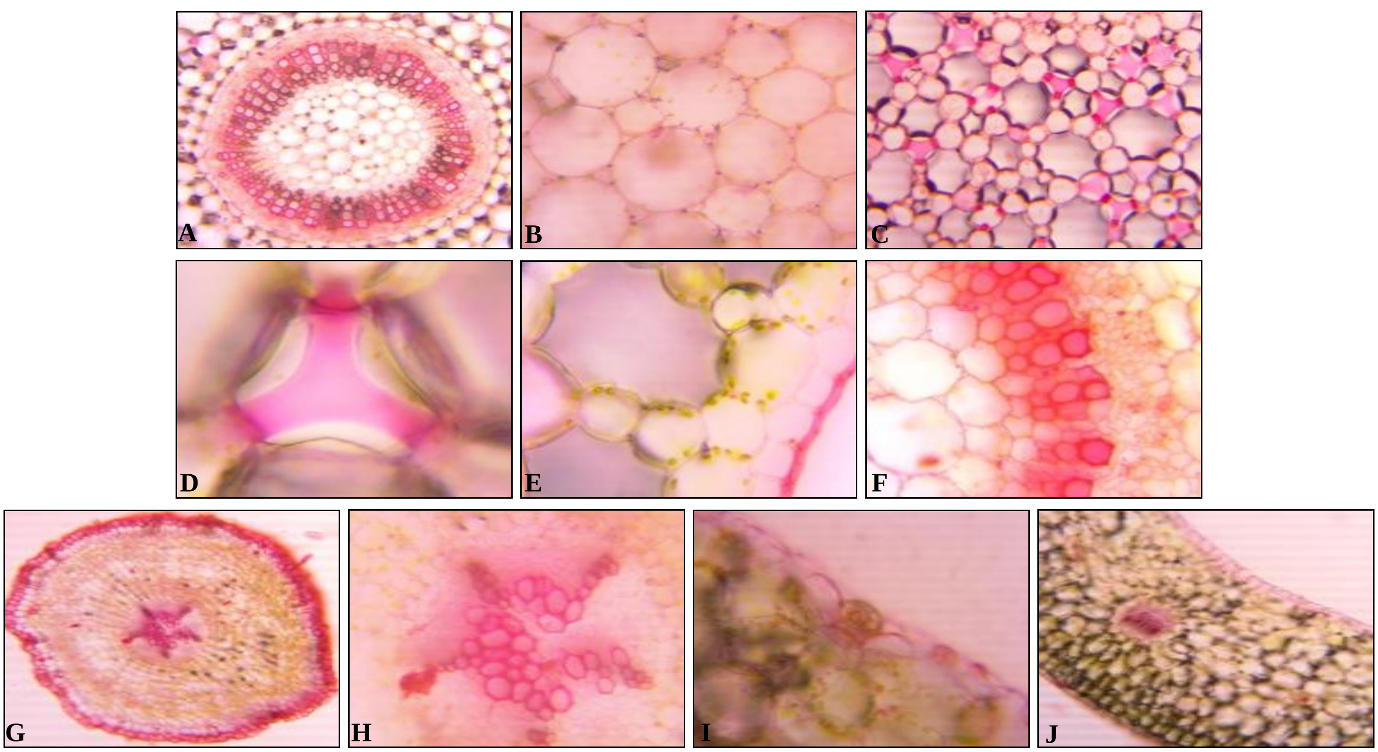


FIG. 2: *BACOPA MONNIERI* (TREATMENT) – A] PHI SHAPED THICKENINGS IN THE ENDODERMIS REGION OF STEM, B] PRISMATIC CRYSTALS OF CALCIUM OXALATE IN THE STEM PITH, C] PARENCHYMATOUS CORTEX WITH Y, X SHAPED CYTOPLASMIC STRANDS, D] ENLARGED VIEW OF SHAPED CYTOPLASMIC STRAND INSIDE PARENCHYMA CELL, E] THICKENED CUTICLE AND STARCH GRANULES IN THE CORTICAL CELLS, F] VASCULAR REGION OF STEM, G] SECTION OF ROOT WITH NUMEROUS STARCH GRANULES, H] ENLARGED VIEW OF VASCULAR REGION OF ROOT, I] SECTION OF LEAF WITH SALT GLAND, J] SECTION OF LEAF SHOWING THE VASCULAR REGION

This is well evident from the presence of salt glands in significantly number in the leaves subjected to salt treatment. Compared to the control, the treatment exhibited a well-thickened cuticle layer above the epidermis of the stem. The hypodermis consisted of parenchyma cells with numerous starch granules in the treatment than in control. The parenchymatous cortex also showed significant adaptive modifications. The presence of numerous peculiar Y, X-shaped cytoplasmic strands inside the parenchyma cells reveals the adaptive tendency of the plant to withstand saline stress conditions. Aerenchyma cells are quiet common in the cortex. The width of the cortex layer was comparatively reduced than the control. Endodermis with phi shaped thickenings was also found in plants subjected to salt treatment. The pith region consists of numerous prismatic crystals of calcium oxalate in control, while it was found only in few numbers in the treatment. Presence of thickened xylem elements in the vascular region of the stem is another adaptive modification. The root showed the presence of starch granules in the cortex region when subjected to salt treatment.

CONCLUSION: Plants encounter elevated levels of essential elements and trace or moderate quantities of toxic, non-essential elements by exhibiting growth retardation, unhinged metabolism or acquiring tolerance mechanisms. General growth retardation is an important established symptom of salt stress in plants. Like other organisms plants also develop mechanisms to tolerate or avoid stressful conditions. Histological modifications are one among the tolerative mechanisms to withstand stress conditions. The plants grown in NaCl produced lignified phi thickenings (Fernandez-Garcia *et al*., 2009) [13]. Even being a glycophytic plant, *Bacopa monnieri* proved potentially fit to grow in saline conditions. The Y, X shaped cytoplasmic strands in the parenchyma cells of the cortex might be an adaptive advantage for the plant to prevent itself from collapsing due to the external saline environment. The morphological and histological changes are therefore a result of mechanisms adopted by the plant to cope up with saline stress conditions.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest regarding the above article.

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