Biological Forum – An International Journal **15(11): 452-455(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Histochemical Studies of Leaf and Stem of *Blepharis sindica* for Localization of Starch and Proteins

Rajneesh Sharma[1,2] and Shikha Roy[1*]
[1]Plant Tissue Culture and Biotechnology Lab, Department of Botany, University of Rajasthan Jaipur (Rajasthan), India.
[2]ICAR-Central Arid Zone Research Institute, Jodhpur (Rajasthan), India.

(Corresponding author: Shikha Roy)*
(Received: 09 September 2023; Revised: 10 October 2023; Accepted: 21 October 2023; Published: 15 November 2023)
(Published by Research Trend)

ABSTRACT: *Blepharis sindica* is vulnerable plant of thar desert which belongs to Acanthaceae family. This plant has typical desert adaptation like narrow leaves, strong root system. Spiny bract to protect the flowers. Because plant has medicinal values due to which localization of different elements (protein, carbohydrates, lipids) in different plant parts like stem ,leaf become important. In stem, protein concentration was observed high in epidermis, outer and inner cortex, pericycle and vascular tissues, xylem and phloem; to some extent it was found in pith. In leaf, concentration of protein was found very high in vascular bundles, bundle sheath, palisade and chlorenchyma tissues and also spongy parenchyma. Upper epidermis and thick walled cells around midrib vascular bundles showed relatively less proteins. In stem, carbohydrates was present predominantly in epidermis, cortex and vascular tissues. However it was relatively less in pith region. In leaf, the carbohydrates was observed in all the tissues of the leaf. It was more prominent in the tissues with thick wall including Upper and Lower epidermis with cuticle. Carbohydrates were more localised in Vascular bundles (Median Vascular bundles) and laminar Vascular bundles, Bundle sheath cells and thick walled tissues and cells around MVb. However, soft tissues like Palisade tissue and Spongy parenchyma was observed with relatively less carbohydrates.

Keywords: Histochemistry, protein, carbohydrates, *Blepharis sindica.*

INTRODUCTION

Blepharis sindica is a herbaceous plant and belongs to Acanthaceae family of Thar desert. The whole plant is used in treatment of several diseases like kidney stone, urine discharge, stomach pain, jaundice, diabetes, rheumatic pain (Mohammed *et al*., 2004). Cream colored seeds are boiled in milk and used as an invigorating tonic. The root of plant is used for urinary discharge and dysmenorrhea (Apurva and Kumari 2015). Powdered plant is applied on infections of the genitals and on burns.

Branch of histology that deals with the identification of chemical component in cells and tissues is known as histochemistry. The histochemical techniques have been employed to characterize structure and development, and to study time course deposition and distribution of major storage compounds such as protein, lipid, starch etc. To determine the localization of proteins and carbohydrates in leaves and stem, tissues of *Blepharis sindica* were subjected to histochemical studies.

Light microscopes developed in 1988 (Ogura *et al.*, 1989) offering less than 2 micron spatial resolution and 25 nanosecond temporal resolution in strobed mode (2 kHz maximum real-time framing rate).

Miranda *et al.* (2017) reported Physiological quality, anatomy and histochemistry during the development of carrot seeds. They reported that it is important to relate physical, anatomical and histochemical characteristics, monitoring the reserves accumulated during seed development, with physiological quality, especially for cultivars of great interest to farmers. Anatomical and Histological Study of Stem, Root and Leaf of the Medicinal Plant *Amaranthus spinosus* Linn has been done by Baril (2018). He reported anatomy and histology as useful tool to understand about the basic features of Plant.

MATERIALS AND METHODS

To study the localization of proteins in leaf and stem, the fresh experimental materials (leaf and stem) were collected from Charan ka bas village (SIKAR district of Rajasthan). To ascertain the localization of proteins and carbohydrates in leaves and stem of tissues of *Blepharis sindica* were subjected to histological studies.

For histochemical studies of proteins the tissues were fixed in carnoys fixative for 12 to 24 hours. They were cleared in benzene, embedded in paraffin and were serially sectioned at 5 to 7 pm thickness. The sections were deparaffinized, washed in absolute alcohol and were stained for 15 minutes in a solution consisting of 1 gm of $HgCl_2$ and 10 gm of bromophenol blue in 10 ml of absolute alcohol. They were first washed in 0.5% acetic acid (with 3 changes) for 20 minutes, then they were briefly washed in water and phosphate buffer (pH 7.0) for 3 minutes. After dehydration in alcohol and

clearing in xylene, the sections were mounted in euparol. Proteins stained green (Pears, 1961; Franceschi *et al*., 1983; Ruthman, 1970).

For carbohydrates the tissues were fixed in Roseman's fluid for 12 to 24 hours. The Roseman's fixed tissues were cleared in benzene, embedded in paraffin and 5 to 7 mm thick serial sections were cut, such sections were deparaffinized, degraded in alcohol grades and were brought to water. The sections were oxidised in 0.5% aqueous periodic acid for four minutes and washed in distilled water. The sections were treated with Schiff's reagent for 15 minutes, washed in running tap water for 10 minutes, dehydrated in alcohol series, cleared in xylene and finally mounted in euparol. The tissues containing polysaccharides stained pink or purple (Pears, 1961; Mc Manus, 1948).

OBSERVATIONS AND RESULTS

1. Protein localization. In stem, protein concentration in stem section seem to be high in epidermis (Ep), outer and inner cortex (Co), pericycle (Pe) and vascular tissues, xylem (Xy) and phloem (Ph); to some extent it is found in pith (Pi); but endodermis (En) seem to be devoid of proteins (Fig. 1A).In leaf, leaf section was observed, concentration of protein was found very high in vascular bundles (Vb), bundle sheath (BS), palisade (Pal) and chlorenchyma tissues and also spongy parenchyma (SP) (Fig. 1C). Upper epidermis (UEp) and thick walled cells around midrib vascular bundles (MVb) showed relatively less proteins (Fig. 1B).

2. Carbohydrate localization. Carbohydrates have been found to be present in all the tissues of the stem as well as leaf only relative amount have found to be variable (Fig. 2). In stem, localization of carbohydrates was observed predominantly in epidermis (Ep), cortex (Co) and vascular tissues in stem section. However it was relatively less in pith region (Fig. 2A). In leaf, the sections of the leaf to study localization of carbohydrates showed its presence in all the tissues of the leaf. It was more prominent in the tissues with thick wall including Upper (UEp) and Lower epidermis (LEp) with cuticle (Cu) (Fig. 2C). Carbohydrates were more localised in Vascular bundles (Median Vascular bundles (MVb) (Fig. 2B) and laminar Vascular bundles (Vb) (Fig. 2C)), Bundle sheath cells (BS) (Fig. 2C) and thick walled tissues and cells around MVb (Fig. 2B). However, soft tissues like Palisade tissue (Pal) and Spongy parenchyma (SP) showed relatively less carbohydrates (Fig. 2C).

DISCUSSION

Thushara and Devipriya (2017) studied histochemical localization of starch, protein, lipid and lignin in the callus, field-grown and *in vitro* raised plants of *Scoparia dulcis* L. They reported that histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major storage compounds such as protein, lipid, starch, phytin and minerals such as calcium, potassium and iron in rice grains.

Blepharis is the only genus in Acanthaceae family which is reported to have C4 species and one of these is *Blepharis ciliaris* (L.) B. L. Burtt., which is a semi-desert species is also have Kranz anatomy (Akhani *et al*., 2008) in which a continuous layer of distinctive bundle sheath cells (Kranz cells) encircle the vascular bundles in cotyledon ,the lateral vascular bundles in leaves and there is extensive development of ground tissue in the midrib and the Kranz tissue becomes interrupted on the abaxial side in older leaves and then becomes completely absent from mature leaf base. *Because Blepharis sindica is a desert plant so it has special anatomical characters leaves has* upper epidermis which is thickly cuticularised. In the stem, epidermis is externally covered by thick cuticle. The epidermal multicellular stem hairs help in protection and heat loss. Hypodermis consists of 3-5 layers of collenchyma without spaces. It contains thickenings by the deposition of extra cellulose with pectin and gives mechanical support.

Choudhary *et al.* (2023) reported presence of carbohydrates, protein and lipid in two species of Blepharis genus (*Blepharis. repens* and other*)* with the help of thin layer chromatography. Because concentration of protein in leaf section observed very high in only median bundle and inner lamina while in stem, concentration of protein seems to be high in epidermis, outer and inner cortex, vascular bundles and pith ,So protein have wider localization cover in stem rather than leaf .Same thing happened with carbohydrate localization. Carbohydrate has distribution in sufficient amount in all over in stem except the pith region but in leaf carbohydrate has efficient distribution in all over except the soft tissues palisade and spongy parenchyma.

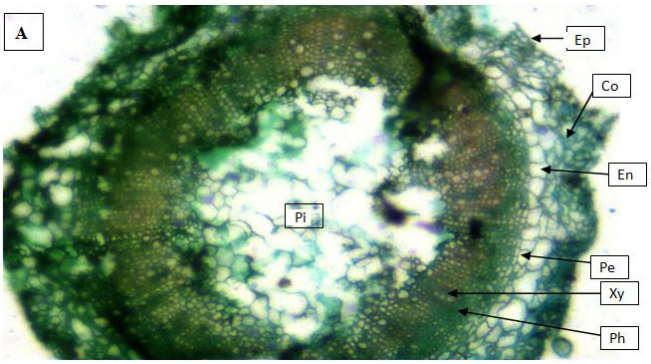

Fig.1. A. Transverse section of stem showing localization of protein in different tissues and cells. Epidermis (Ep), Cortex (Co), Endodermis (En), Pericycle (Pe), Xylem (Xy), Phloem (Ph) and Pith (Pi).

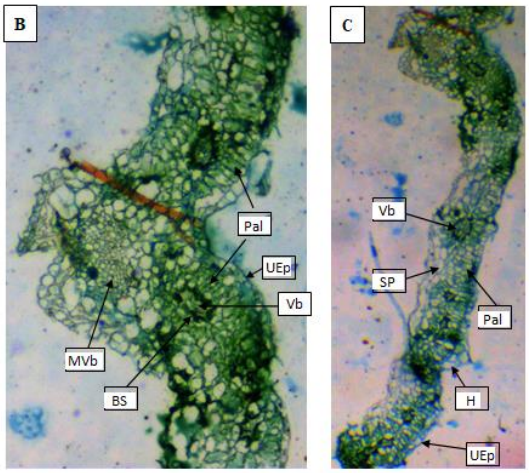

Fig.1. B & C. T.S. of leaf midrib region and lamina showing distribution of protein in different tissues and cells. Upper epidermis (UEp), Palisade tissue (Pal), Vascular bundle (Vb), Bundle sheath (BS), Midrib vascular bundle (MVb), Spongy parenchyma (SP) and Hair (H).

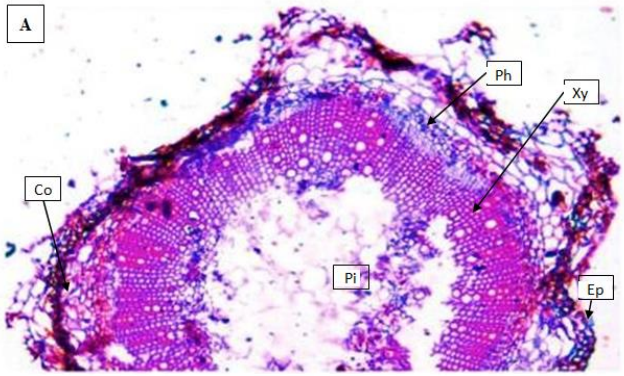

Fig. 2. A. Transverse section of stem showing localisation of carbohydrates in different tissues and cells. Epidermis (Ep), Cortex (Co), Phloem (Ph), Xylem (Xy) and Pith (Pi).

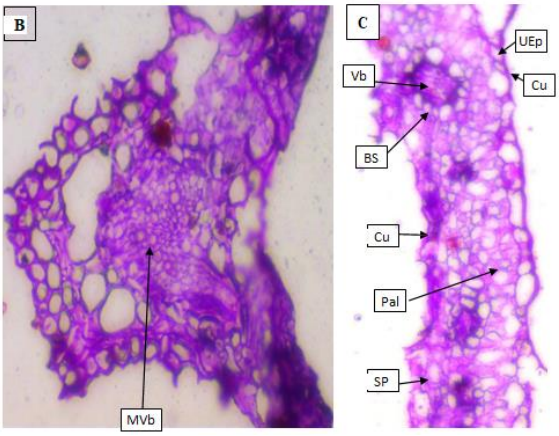

Fig. 2. B & C. T.S. of leaf midrib region and lamina showing distribution of Carbohydrates in different tissues and cells. Upper epidermis (UEp), Cuticle (Cu), Palisade tissue (Pal), Vascular bundle (Vb), Bundle sheath (BS), Spongy parenchyma (SP), and Midrib vascular bundle (MVb).

CONCLUSIONS

It is concluded that *Blepharis sindica* have proteins and carbohydrate presence in the different layer of stem and leaf. In leaf, very high concentration of protein in only median bundle and inner lamina but in case of stem high concentration of protein is in epidermis, outer and inner cortex, vascular bundles and pith so stem has large distribution area for protein as compare to leaf. Carbohydrate has distribution in sufficient amount in all over in stem except the pith region but in leaf carbohydrate has efficient distribution in all over except the soft tissues like; palisade and spongy parenchyma.

FUTURE SCOPE

Lipid localization studies can be carried out in future for this plant. Root part is not covered here so it can be covered for these localization studies of proteins, carbohydrates and lipid in future.

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How to cite this article: Rajneesh Sharma and Shikha Roy (2023). Histochemical Studies of Leaf and Stem of *Blepharis sindica* for Localization of Starch and Proteins. *Biological Forum – An International Journal*, *15*(11): 452-455.