J Biosci (2021)46:51
DOI: 10.1007/s12038-021-00174-2

A microscopic study on scattering in tissue section of *Alternanthera philoxeroides* under polarized light

Shibsankar Roy[1], Barnini Bhattacharya[1,2], Bijay Bal[1,3] and Kuntal Ghosh[1,4]*

[1]*Laboratory for Cognitive Systems and Cybernetics Research, Center for Soft Computing Research, Indian Statistical Institute, Kolkata 700 108, India*

[2]*Department of Physiology, University of Calcutta, Kolkata, India*

[3]*Saha Institute of Nuclear Physics (Retired), Kolkata, India*

[4]*Machine Intelligence Unit, Indian Statistical Institute, Kolkata, India*

**Corresponding author (Email, kuntal@isical.ac.in)*

MS received 25 August 2020; accepted 8 May 2021

Like any other biological tissue, plant tissue also exhibits optical properties like refraction, transmission, absorption, coloration, scattering and so on. Several studies have been conducted using different parts of plants such as leaves, seedlings, roots, stems and so on, and their optical properties have been analyzed to study plant physiology, influence of environmental cues on plant metabolism, light propagation through plant parts and the like. Thus, it is essential to study in detail the optical properties of several plant parts to determine their structural relationship. In this backdrop, an experimental study was conducted to observe and analyze the optical properties of node and inter-nodal tissue cross-sections of the plant *Alternanthera philoxeroides* under a polarizing microscope constructed and standardized in the laboratory. The observed optical properties of the microscopic tissue sections have been then studied to determine a significant structural relationship between nodal and inter-nodal tissue arrangement patterns as a whole. Tissue sections that have undergone a sort of biological perturbation like loss of water (dried in air for 15 min) have also been studied to study the change in the pattern of tissue optical property when compared with that of normal plant-tissue cross-sections under a polarizing microscope. This type of biological perturbation was chosen for the study because water plays an important role in maintenance of the normal physiological processes in plants and most other forms of life.

Keywords. *Alternanthera philoxeroides*; inter-node; node; polarizing microscopy; scattering; tissue section

1. Introduction

The optical properties of a tissue can be described in terms of absorption coefficient, scattering coefficient, scattering function, deflection angle of scatter and refractive index of the tissue (Jacques 2013). Biological tissues are optically inhomogeneous and absorbing media whose average refractive index is higher than that of air (Tuchin 2007). Like other biological tissues, plant tissues (both, microstructures as well as macrostructures) also exhibit optical properties like refraction, absorption, coloration, scattering and so on (Lee 2009). Such properties, in turn, depend on certain tissue characteristics like type and shape of the tissue, tissue-density and thickness, and tissue structure, and therefore, the exhibited optical properties are not uniform throughout a tissue section (when viewed under a microscope) and vary from region to region (Chance *et al*. 1998; Ansari and Mohajerani 2011; Preuss and Profio 1989). Leaves are one of the important optical organs with complex tissue organization (Vogelmann 1993). For this reason, leaf optics has been used enormously by scientists worldwide in studying plant physiology, remote sensing and the like (Tucker and Sellers 1986; Jacquemoud *et al*. 1996; Datt 1999). Previous studies have shown that it is essential to understand the optical properties of plant tissues to comprehend their structure–function relationship as

Published online: 09 June 2021

well (Lee 2009; Mandoli and Briggs 1982; Esau 1977; Fahn 1990). Some studies have been carried out on plant leaves and seedlings to investigate the influence of plant tissues on incident light. Other studies have been conducted to record the internal axial light conduction in roots and stems of plants (Sun *et al*. 2004). However, using polarizing microscopy to observe and analyze tissue optical properties of cross-section of *Alternanthera philoxeroides* plant segments is not very well documented.

Polarized light microscope is a type of optical microscope that is designed to analyze and photograph specimens that are visible primarily due to their optically anisotropic character and exhibiting self-polarization property (Oldenbourg 2013). A polarizing microscope consists of a polarizer and analyzer. The polarizer is placed below the sample that allows specific vibrational direction of light's electric field to pass through it. The analyzer is placed above the sample that detects the change in direction of vibration (thereby illuminating the sample). In this backdrop, the optical properties of the tissue have been analyzed in-depth using an optimized optical arrangement under a polarized microscopic setup (designed in the laboratory), and such properties have been utilized, in turn, to find out their significant structural correlation at microscopic scale. Tissue sections that have undergone a sort of biological perturbation like water deprivation (dried in air for 15 min) have also been studied to determine the effect of water loss on the plant-tissue optical property under the polarizing microscope when compared with that of normal plant-tissue cross-sections. This type of biological perturbation was chosen for the study because water plays an important role in maintenance of the normal physiological processes in plants and most other forms of life.

2. Materials and methods

Alternanthera philoxeroides were collected from campus (Indian Statistical Institute, Kolkata) garden before obtaining the microscopic sections. A total of 6 plants, of more or less similar dimensions, were used for the study. For preparation of the cross-section of stem (order of 170–250 μm) a microtome (constructed in the laboratory), a digital polarizing microscope (constructed in the laboratory), slide, mountant and coverslips were used. The polarizing microscope was connected to the computer display for image capturing. The cross-sections were viewed under two modes – plane polarized mode, where the polarizer and analyzer were parallel to each other, and cross-polarized mode, where the analyzer was at a particular angle to that of the polarizer. Based on the optical property of the tissue sections and for detection of changes in the pattern of optical property of the perturbed tissue cross-sections, the cross-polarized mode was further divided into three sub-ranges: first appearance range I (45° to 70°), first appearance range II (71° to 85°) and maximum contrast range (86° to 90°). Two positions of the stem were chosen for structural analysis of the tissue cross-sections: one at node and the other at inter-nodal fragment. To detect any change in pattern of tissue optical property as a result of a biological perturbation under polarizing microscope, at first, a normal plant tissue section was observed under the microscope and then that same tissue section was kept under water loss condition (dried in air for 15 min) and viewed under the polarizing microscope. To determine whether this type of perturbation was reversible or not, those affected (under water loss condition) tissue cross-sections were rewatered (immersed in water medium for 15 min) and similarly viewed under the microscope.

3. Results

After observation and analysis of optical properties of the tissue cross-sections of stem of *A. philoxeroides* under the polarizing microscope, it was seen that the tissues exhibit scattering and color absorption property. The characteristic features of the normal, water-deprived and rewatered tissue cross-sections under the four different viewing modes of the microscope have been also studied and compared (table 1).

Plane-polarized mode: In plane-polarized mode (analyzer and polarizer parallel to each other), the intensity of the light passing through the tissue with respect to that of background was difficult to differentiate at certain areas of the section. In the case of the tissue section of the inter-nodal segment (figure 1(a)), the vascular bundle was visible but it was difficult to differentiate between xylem, phloem and pericycle. The difference between filled vacuoles and gas vacuoles was also not prominent. The pigment distribution within tissues was not clear. In the case of the tissue section of the node region (figure 1(b)), only the cortex and vascular bundle were visible. In the case of the inter-nodal tissue section under water-deprived condition (figure 2(b)), the epidermis was seen to have undergone slight deformity. The tissues of the cortical region also showed slight shrinking and the cortical space between epidermis and cambium ring

Table 1. Comparative characteristic features among normal, water-deprived and rewatered tissue cross-sections of the inter-nodal region when viewed under the different modes of the polarizing microscope

Viewing modes	Normal	Water-deprived	Rewatered
Plane polarized mode	Epidermis, interfascicular cambium and cortical tissues appeared normal in shape, i.e., without any deformity (figure 2(a))	>Epidermis was deformed >Slight shrinkage of the tissues of the cortical region >Minute reduction in the cortical space between epidermis and interfascicular cambium (figure 2(b))	Epidermis and tissues of cortical region regained their structural appearances (like that of normal section) (figure 2(c))
First appearance range I (45° to 70°)	Epidermis, interfascicular cambium and pericycle were the first to show the scattering property (figure 2(d))	No initial scattering of the epidermis, interfascicular cambium and pericycle observed (figure 2(e))	First scattering of the epidermis, interfascicular cambium and pericycle were again visible (figure 2(f))
First appearance range II (71° to 85°)	Scattering intensities of the epidermis, interfascicular cambium and pericycle increased (figure 2(g))	The initiation of scattering property of the epidermis, interfascicular cambium and pericycle started to become visible (figure 2(h))	The scattering intensities of the epidermis, interfascicular cambium and pericycle were similar to that of the normal tissue section (figure 2(i))
Maximum contrast range (86° to 90°)	Maximum scattering intensities of the epidermis, interfascicular cambium and pericycle (figure 2(j))	Marked reduction in the relative scattering intensities of the epidermis, interfascicular cambium and pericycle (figure 2(k))	The overall scattering intensity again increased (like that of normal state) (figure 2 (l))

(interfascicular cambium) appeared to have reduced a bit. However, when the same tissue section was rewatered (figure 2(c)), the epidermis and cortical region regained their structural appearances, like that of the normal tissue section (figure 2(a)).

Cross-polarized modes:

First appearance range I (45° to 70°) – When the polarizer was between 45° to 70° with respect to analyzer (vibrational direction of electric component of light wave was between 45° to 70°), the relative intensity became slightly distinguishable, and therefore, under this particular arrangement, the tissues with maximum scattering property were the first to become visible. In this mode, in case of plant-tissue section of inter-nodal segment (figure 1(c)), the epidermis, interfascicular cambium and pericycle (bundle cap) were the first to show the scattering property; pigmentation appeared greener but the cortical tissue layers lost its prominence. In the case of the plant-tissue section of the node region (figure 1(d)) the cortex, green pigments and future leaf region were very clear but the vascular bundle was not so prominent. In the tissue sections under water-deprived condition (figure 2(e)), no initial scattering of the epidermis, interfascicular cambium and pericycle were visible when viewed under this particular mode. But when the same tissue cross-section was rewatered (figure 2(f)) for 15 min and viewed under this mode, the first scattering of epidermis and interfascicular cambium were again prominent, similar to that of the normal tissue cross-section (figure 2(d)).

First appearance range II (71° to 85°) – In this particular mode, the above-mentioned kind of biological perturbation in the plant-tissue cross-sections became detectable. It was observed that in the case of tissue cross-sections under water-deprived condition (figure 2(h)), the first appearance of tissue scattering property was detected in this range in comparison to the normal tissue cross-sections, where initial appearance of scattering was observed in the First appearance range I (45° to 70°). The deformity of the structure of the cambium ring was quite prominent under this mode in comparison to the image viewed under plane polarized mode and first appearance range I – cross-polarized mode.

Maximum contrast range (86° to 90°) – Under this mode, when the polarizer was between 86° to 90° (vibrational direction of electric component of light wave was between 86° to 90°), the relative intensity

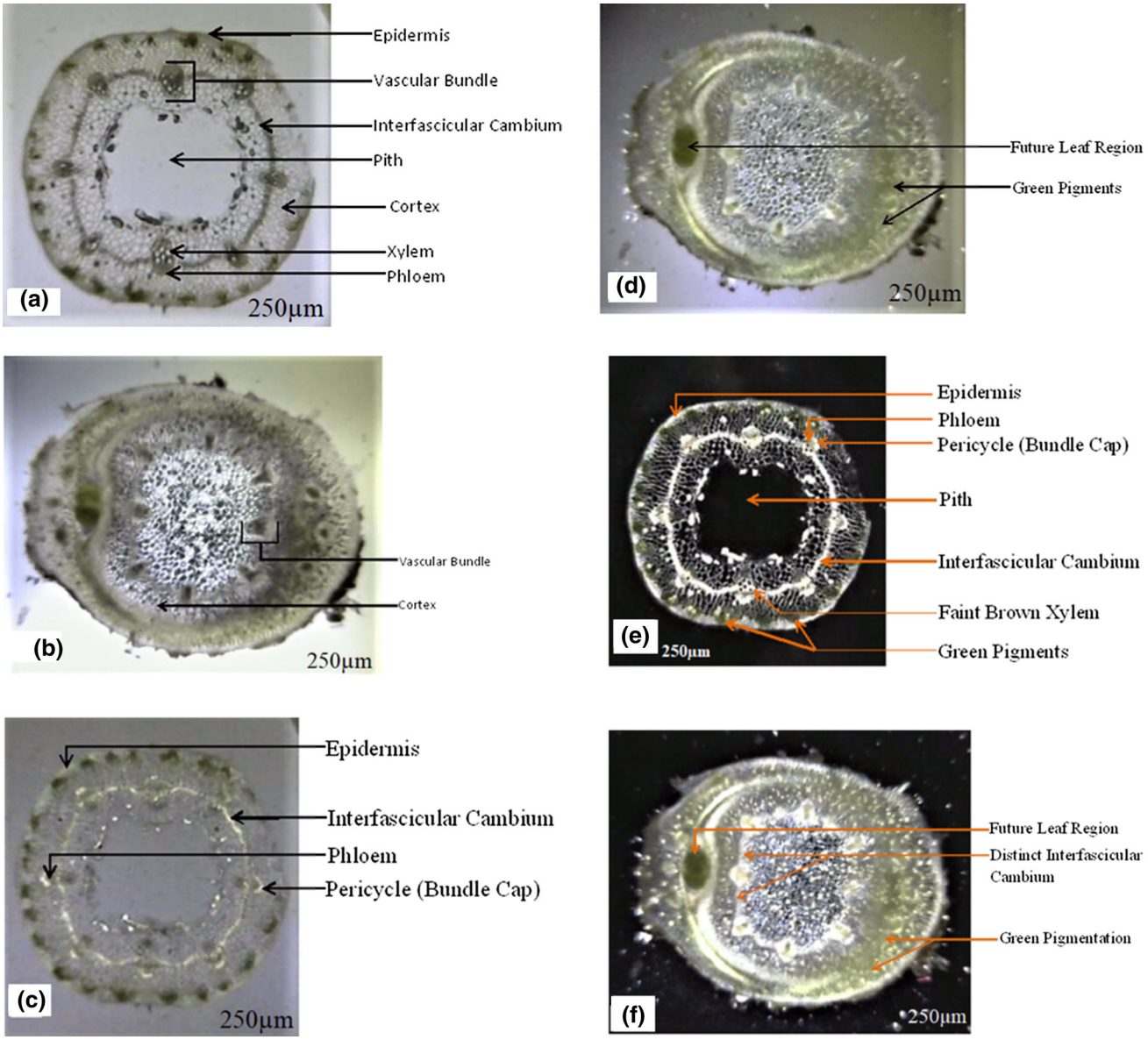

Figure 1. Microscopic images of tissue cross-sections of *A.philoxeroides* (section thickness in the order of 250 µm). (**a**) Inter-nodal cross-section under Plane Polarized Mode. (**b**) Nodal cross-section under Plane Polarized Mode. (**c**) Inter-nodal cross-section under First Appearance Range I (45° to 70°) of Cross-Polarized Mode. (**d**) Nodal cross-section under First Appearance Range I (45° to 70°) of Cross-Polarized Mode. (**e**) Inter-nodal cross-section under Maximum Contrast Range (86° to 90°) of Cross-Polarized Mode. (**f**) Nodal cross-section under Maximum Contrast Range (86° to 90°) of Cross-Polarized Mode.

was maximally distinguishable, and thus under this arrangement even tissues with minimum scattering property were also markedly visible. In the tissue section of the inter-nodal segment (figure 1(e)), the vascular bundle was prominent; xylem, phloem and pericycle were markedly distinct; xylem appeared to be faint brown. It was seen that pericycle, interfascicular cambium and epidermis appeared to be whitish (more prominent scattering); green pigment was also prominent. The cortical tissue layers also showed scattering but not like the others; gas and filled vacuoles were distinctly differentiable – filled containing vacuoles appeared whitish. The pith was quantitatively conspicuous. In case of nodal region tissue section (figure 1(f)), even though the cortex became denser and pith got filled up, the interfascicular cambium was highly prominent; green pigments and white-droplet-like structures distributed throughout the section were also distinguishable from each other, unlike at plane polarization mode and first appearance range I and II of

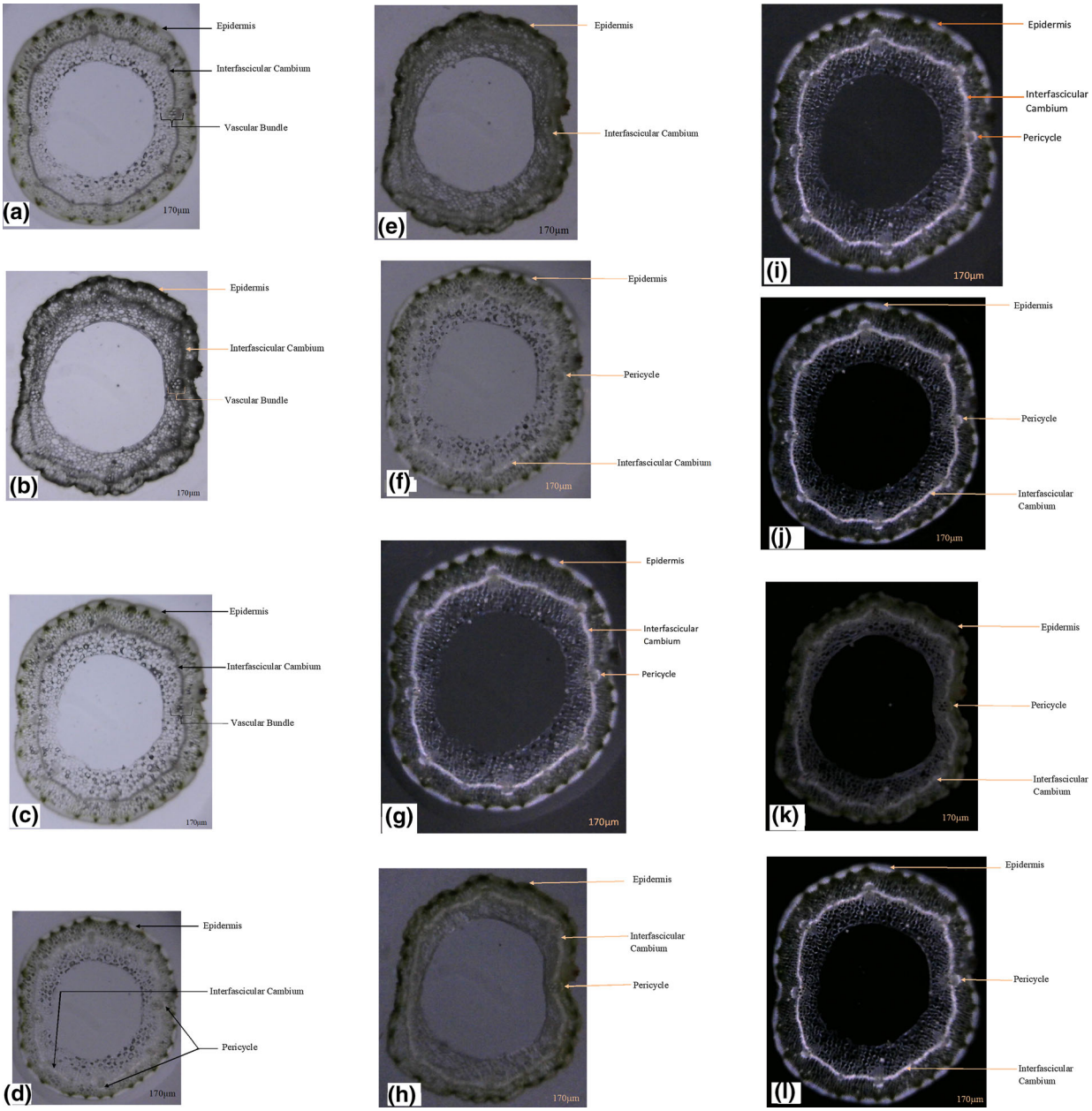

Figure 2. Microscopic images of inter-nodal tissue cross-section under normal, water-deprived and rewatered conditions (section thickness in the order of 170 µm). (**a**) Normal (plane polarized) – Epidermis, interfascicular cambium and cortical tissues without any deformity. (**b**) Water-deprived (plane polarized) – Slight deformation of epidermis and shrinkage of cortical tissues. (**c**) Rewatered (plane polarized) – Structural appearances restored to normal. (**d**) Normal (first appearance range I – 45° to 70°) – First scattering of epidermis, interfascicular cambium and pericycle became visible. (**e**) Water-deprived (first appearance range I – 45° to 70°) – No initial scattering of epidermis, interfascicular cambium and pericycle observed. (**f**) Rewatered (first appearance range I – 45° to 70°) – First scattering of epidermis, interfascicular cambium and pericycle reappeared. (**g**) Normal (first appearance range II – 71° to 85°) – Increase in scattering intensities of epidermis, interfascicular cambium and pericycle. (**h**) Water-deprived (first appearance range II – 71° to 85°) – First scattering of epidermis, interfascicular cambium and pericycle started to become visible under this range. (**i**) Rewatered (first appearance range II – 71° to 85°) – Scattering intensities of epidermis, interfascicular cambium and pericycle similar to normal cross-section. (**j**) Normal (maximum contrast range – 86° to 90°) – Maximum scattering intensities of epidermis, interfascicular cambium and pericycle. (**k**) Water-deprived (maximum contrast range – 86°to 90°) – Marked reduction in relative scattering intensities of epidermis, interfascicular cambium and pericycle. (**l**) Rewatered (maximum contrast range – 86° to 90°) – overall scattering intensity again increased.

cross-polarization mode. The future leaf portion (green elliptical structure) was also very clear and appeared as a region separating out from the cortex. In case of the tissue cross-section under water-deprived condition (figure 2(k)) the overall scattering intensity of the tissue regions that once showed maximum scattering under normal condition appeared to have reduced relatively. However, when the same cross-section was rewatered (figure 2(l)) for 15 min, the overall scattering intensity appeared to have increased (apparently returned to normal state), similar to that of the normal tissue cross-section (figure 2(j)).

4. Discussion

Scattering of light is the phenomenon by which a directional beam of light is redirected in many different directions when it interacts with wavelength-matching particles (Kerker 2013). In polarizing microscope by changing the relationship of the polarizer and the analyzer, it is possible to determine the amount of absorbance, scattering, reflection and refraction of the light through the sample (Oldenbourg 2013). In cross-polarization mode only samples having their own polarization property become visible, when placed at a particular angle with respect to the optical axis. That is why, upon rotation, the sample becomes visible only at a particular angle and not the other. The incident light that is projected onto the sample is a one-state polarized light (by passing through polarizer). When an anisotropic sample, like that of cross-section of plant tissue was placed in path of the one state polarized light, a part of the polarized light got redirected into multi-direction due to difference in refractive indices of the successive layers of the medium and resulted in absorption of some selective wavelengths and random scattering of light from selective part of whole tissue, while the light coming from the background (free of tissue parts) was still maintained at the incident state of polarization (Kerker 2013). When the analyzer was kept at 86°–90° (with respect to polarizer) the directional background light was blocked but some part of the randomly scattered (multidirectional) light coming from the sample passed through the analyzer at all angles of rotation. This resulted in the formation of a contrast between the sample and background. It is known that, the higher the tissue density, the higher is its rate of random scattering with respect to its background. Contrast may be defined as the difference in color and brightness properties of an object that makes it distinguishable from other objects and the background. It results from the difference in luminance reflected from two adjacent surfaces. Mathematically, there are various possible definitions of contrast like Weber Contrast, Michelson Contrast and so on. In the present context of study of tissue properties, the Michelson formulation will be an appropriate choice (Rühr and Lambertz 2019). Light that passed through free spaces surrounding the tissue cross-section constituted the background light and the electric field of this background light remained similar to that of the incident light.

In this study, as mentioned previously, based on the tissue optical property, the cross-polarization mode was divided into three sub-ranges: (first appearance range I (45° to 70°), first appearance range II (71° to 85°) and maximum contrast range (86° to 90°)). On the basis of such three sub-ranges, the respective values of background intensity and contrast may be calculated as follows:

When analyzer is at an angle θ with polarizer, the electric field component of background in direction of analyzer will be $E \cos \theta$ and the corresponding intensity of light will be $E^2 \cos^2 \theta$. For example, when analyzer and polarizer is at angle 45° to each other the intensity of background light will be

$$E^2(\cos 45)^2 = E^2/2.$$

If E^2 is replaced by I, then the intensity of background light becomes $I/2$. Consequently, when the analyzer is at 90° to that of the polarizer, the intensity of background light becomes zero and is thus expected to be minimum. As mentioned previously, Michelson Contrast (C) is expressed as,

$$\frac{I_{max} - I_{min}}{I_{max} + I_{min}},$$

where I_{max} and I_{min} represent highest and lowest luminance. So under the cross-polarization 45° to 70° mode (first appearance range I), the contrast C of background (background intensity) is reduced between 0.3 and 0.1; in the cross-polarization 71° to 85° mode (first appearance range II), the contrast C of background (background intensity) is reduced between 0.1 and 0.02; and under the 86° to 90° cross-polarization mode (maximum contrast range), the background intensity is reduced between 0.02 to 1. These minute differences among the three sub-ranges of the cross-polarization mode were also evident to the human eye based on the tissue cross-sectional images (figure 1 and 2). Due to presence of pigment in tissue, certain wavelengths of light were allowed to pass and others

got absorbed and this resulted in the appearance of certain colors. This appearance of color of pigmented tissues and that of xylem were due to selective absorption: green pigmentation appeared due to absorption of red and blue color and the faint brownish appearance of xylem was due to partial absorption of green color. In cross-polarization mode, upon rotation of the slide containing the sample, the observed features remained unchanged with respect to the previously mentioned modes where the sample was kept fixed. This feature indicates that the plane polarized light, which was partially absorbed in the parts of the tissue, was not subsequently emitted in a particular direction but was rather, in a randomly scattered direction. This means that during the passage of plane polarized light through the sample a part of the light was absorbed and re-emitted in a slightly random direction in the visible plane. As a result of such random scattering, some of the components of the re-emitted light coming out of the sample were always available in the visible plane of the image and thus, no noticeable change in the features of the image was observed even after rotation of the sample. Thus, it provides an important clue for imaging of different parts of biological tissues with enhanced relative contrast.

In the study after comparing (table 1) the characteristic features of the inter-nodal tissue cross-sections under normal, water-deprived and rewatered conditions when viewed under the different modes of the polarizing microscope it was evident that following a 15min duration of water deprivation, there was a marked reduction in the scattering intensities of the epidermis, interfascicular cambium and pericycle in comparison to the normal and rewatered tissue cross-section (figure 2). This notable reduction in scattering intensity following loss of water in the tissue cross-section was detected under the three sub-ranges: first appearance range I (45° to 70°), first appearance range II (71° to 85°) and maximum contrast range (86° to 90°) of the cross-polarization mode of the microscope. In 2017; Hochberg *et al.* performed an experimental study on grapevine plant to continuously monitor xylem cavitation and flow rates in the stem of an intact and dehydrated plant using magnetic resonance imaging (MRI). The imaging results of the study conducted by Hochberg *et al.* indicated that water filled xylem vessels appeared brighter and gas-filled xylem vessels appeared black. In the water volume flow map, under high water flow rate condition, the pixels were brighter and conversely, under low flow rate condition the pixels were darker (Hochberg *et al.* 2017). The type of biological perturbation chosen for the present study was water deprivation for 15min. To detect whether this type of perturbation was reversible or not the tissue sections were again watered for 15 min and observed under the polarizing microscope. From the image results (figure 2(f) and 2(i)) it was observed that instead of the shift in the initial scattering from first appearance range I to II as seen in case of the dried (perturbed) tissue cross-sections, the occurrence of initial scattering in the rewatered cross-sections returned to the first appearance range I (similar to normal tissue cross-sections). The overall intensity of the tissue scattering also increased to normal level (figure 2(l)). This may be indicative of a reversible biological perturbation. In this study, it was important to significantly correlate the tissue optical properties and their structural arrangement patterns, as a whole and not that of cells. For this reason, the magnification range was kept within 25× to 50× so that the whole tissue pattern of the cross-section becomes visible under the same microscopic field of view.

5. Conclusion

From the present study it has been found that scattering and color absorption occurs in tissue cross-sections of the nodal and inter-nodal segments of the plant *A. philoxeroides* when viewed under different modes of polarizing light microscopy. The optical properties of the tissue cross-sections have been assessed to determine the significant relationship between the whole tissue structural arrangement pattern of nodes and that of inter-node segments of the plant. Analysis of region-specific (node and inter-node) whole tissue architecture may, in turn, help to determine the structure-function relationship at specified locations within the plant tissues. After studying the characteristic features of the images under the different conditions (normal, water loss and rewatered), it was seen that water deprivation reduces the overall intensity of the microscopic tissue scattering. Thus, using a polarizing microscope, biological perturbations such as water loss in microscopic plant tissue cross-sections may be detected.

Acknowledgements

This project is funded by Cognitive Science Research Initiative (CSRI) of Department of Science and Technology, India. The authors remain grateful to faculty

members of the Biological Sciences Division of Indian Statistical Institute, Kolkata.

References

Ansari MA and Mohajerani E 2011 Mechanisms of laser-tissue interaction: optical properties of tissue. *Lasers Med. Sci.* **2** 119–125

Chance B, Cope M, Gratton E, Ramanujam N and Tromberg B 1998 Phase measurement of light absorption and scatter in human tissue. *Rev. Sci. Instrum.* **693** 457–3481

Datt B 1999 A new reflectance index for remote sensing of chlorophyll content in higher plants: tests using Eucalyptus leaves. *J. Plant Physiol.* **154** 30–36

Esau K 1977 *Anatomy of Seed Plants* (New York: J Wiley & Sons)

Fahn A 1990 *Plant Anatomy* (United Kingdom: Oxford Press)

Hochberg U, Windt CW, Ponomarenko A, Zhang YJ, Gersony J, Rockwell FE and Holbrook NM 2017 Stomatal closure, basal leaf embolism, and shedding protect the hydraulic integrity of grape stems. *Plant Physiol.* **174** 764–775

Jacquemoud S, Ustin SL, Verdebout J, Schmuck G, Andreoli G and Hosgood B 1996 Estimating leaf biochemistry using the PROSPECT leaf optical properties model.*Remote Sens. Environ.* **56** 194–202

Jacques SL 2013 Optical properties of biological tissues: a review. *Phys. Med. Biol.* **58** 37–61

Kerker M 2013 Introduction; in *The scattering of Light and other Electromagnetic Radiation: Physical Chemistry: A Series of Monographs* (ed) Loebl ME (United States of America: Academic Press)

Lee DW 2009 Plant tissue optics: micro-and nanostructures. In Biomimetics and Bioinspiration **7401** 740104. International Society for Optics and Photonics

Mandoli DF and Briggs WR 1982 Optical properties of etiolated plant tissues. *Proc. Natl. Acad. Sci.* **79** 2902–2906

Oldenbourg R 2013 Polarized light microscopy: principles and practice. *Cold Spring Harb. Protoc.* **11** 1023–1036

Preuss LE and Profio AE 1989 Optical properties of mammalian tissue: introduction by the feature editors. *Appl. Opt.* **28** 2207–2209

Rühr PT and Lambertz M 2019 Surface contrast enhancement of integumentary structures in X-ray tomography. *J. Anat.* **235** 379–385

Sun Q, Yoda K and Suzuki H 2004 Internal axial light conduction in the stems and roots of herbaceous plants. *J. Exp. Bot.* **56** 191–203

Tuchin VV 2007 *Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis* (Washington: Society of Photo-Optical Instrumentation Engineers)

Tucker CJ and Sellers PJ 1986 Satellite remote sensing of primary production. *Int. J. Remote Sens.* **7** 1395–1416

Vogelmann TC 1993 Plant tissue optics. *Annu. Rev. Plant Biol.* **44** 231–251

Corresponding editor: BJ RAO