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Imaging linear and circular polarization features in leaves with complete Mueller matrix polarimetry

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ABSTRACT

Spectropolarimetry of intact plant leaves allows to probe the molecular architecture of vegetation photosynthesis in a non-invasive and non-destructive way and, as such, can offer a wealth of physiological information. In addition to the molecular signals due to the photosynthetic machinery, the cell structure and its arrangement within a leaf can create and modify polarization signals. Using Mueller matrix polarimetry with rotating retarder modulation, we have visualized spatial variations in polarization in transmission around the chlorophyll a absorbance band from 650 nm to 710 nm. We show linear and circular polarization measurements of maple leaves and cultivated maize leaves and discuss the corresponding Mueller matrices and the Mueller matrix decompositions, which show distinct features in diattenuation, polarizance, retardance and depolarization. Importantly, while normal leaf tissue shows a typical split signal with both a negative and a positive peak in the induced fractional circular polarization and circular dichroism, the signals close to the veins only display a negative band. The results are similar to the negative band as reported earlier for single macrodomains. We discuss the possible role of the chloroplast orientation around the veins as a cause of this phenomenon. Systematic artefacts are ruled out as three independent measurements by different instruments gave similar results. These results provide better insight into circular polarization measurements on whole leaves and options for vegetation remote sensing using circular polarization.

1. Introduction

One of the most distinctive and characteristic features of life is the homochirality of its molecular building blocks [1]. Chiral molecules in their most simple form exist in left-handed (L-) and a right-handed (D-) versions, called enantiomers. In non-biological systems, the mixture is expected to be racemic (50%–50 %). However, biological systems tend to have nearly 100% preference for one type of enantiomer, which is a feature called homochirality. In fact, the functioning and structure of biological systems is largely determined by their chiral constituents. Although there are a few exceptions [2], amino acids mainly occur in the L-configuration and sugars occur predominantly in the D-configuration. Apart from these small molecules, many large scale molecular architectures, dimensions of which can range over several orders of magnitude, are chiral. An example of such large-scale chirality is displayed by the DNA molecule, which is always right-handed and can be over 2 m long [3]. Chirality can also be observed in the chlorophylls and bacteriochlorophylls, in particular when utilized in photosynthesis (as their intrinsic signal is very weak due to their planar and almost symmetrical structure). Additionally, these chlorophylls are organized

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in a supramolecular structure that itself is chiral too [4].

The molecular dissymmetry of chiral molecules has a specific response to electromagnetic radiation [5] and this response both depends on the intrinsic chirality of the molecules and on the chirality of the supramolecular architecture. Examples of available spectroscopic methods that are based on this interaction are circular diattenuation (dichroism) and linear diattenuation spectroscopy. Both methods are complementary and offer valuable insight into the functionality and structure of molecules and have a long history in the research on photosynthesis [4]. In circular dichroism spectroscopy, the differential extinction of left- and right-handed circularly polarized light as a function of wavelength is measured. Linear diattenuation spectroscopy characterizes the change in extinction depending on the linear polarization of the incident (orthogonal) beams. Usually, only isolated molecules or cell constituents are measured, but it has recently been shown that the circular dichroism of whole leaves can also be determined [6, 7]. This is not possible in linear diattenuation spectroscopy, since the retrieval of structural information is dependent on the molecular alignment of the sample. In a randomly oriented sample, such as in a leaf, this information is therefore averaged out.

Mueller matrix polarimetry allows a thorough characterization of the polarization properties of a sample. The complete Mueller matrix is a 4×4 matrix with real elements that completely describes the polarization response of an optical element. Within its elements it additionally contains polarization properties, i.e., circular and linear diattenuation, retardance, and depolarization. Diattenuation is similar to dichroism, although the latter is usually described in terms of absorbance. The retardance describes the phase changes of light and is independent of the intensity transmittance. The depolarization describes the ratio of incident light that becomes unpolarized upon interaction with the sample. The mathematical descriptions of these quantities will be given below.

Both linear and circular dichroism spectroscopy depend on the modulation of the incident light and the subsequent differential interaction within the sample resulting in a measurable difference. Induced linear polarization is also measurable and scattered linear polarization has been investigated for vegetation remote sensing [8,9,10,11,12]. Although it has been suggested that linear polarization remote sensing offers no additional information compared to the scalar reflectance [8], it has recently been suggested that it could be a promising remote sensing tool for the detection of leaf structural changes such as brought upon by drought [11].

Also circular polarization by photosynthetic systems might potentially be a powerful tool for the remote sensing of biosignatures on Earth and beyond [13,14,15]. Recently, it has been shown that the induced fractional circular polarization by phototrophic organisms can be measured successfully in detail [7, 16, 17] and is comparable to circular dichroism measurements [7]. Unlike linear spectropolarimetry, circular spectropolarimetric measurements still contain the structural information resulting from the chiral molecular systems. As such, scattered circular polarization might prove to be both a unique remotely applicable tool for vegetation monitoring on Earth as well as a powerful remotely accessible means of detecting the unambiguous presence of extraterrestrial life.

Nonetheless, relatively few *in vivo* induced circular polarization studies on phototrophic organisms are available. We previously showed that the amount of induced circular polarization of unpolarized light is equivalent to the differential absorbance of incident circularly polarized light for *in vivo* transmission measurements on leaves [7]. These results are evidence for at least a general cross sectional isotropy in the fractional circular polarizing/absorbing component. Little, however, is known about the possible spatial variation in the polarizing components of leaves which can offer more information about the origin of the polarization signals.

Depending on the area of the leaf that is measured, such spatial variations might lead to inaccuracies if the molecular architecture is investigated. This is especially important for *in vivo* measurements on leaves carried out using commercial dichrographs (due to the relatively small area of measurement) and it might also be important to consider when scaling up fractional polarization measurements to remote sensing applications.

The typical circular polarization signal observed from chloroplasts is the result of the superposition of two relatively independent signals resulting from different chiral macrodomains [18]. These result in bands of opposite sign that do not have the exact same spectral shape and thus do not cancel each other out completely. The existence of these macrodomains was first demonstrated using differential circular polarization scattering [19] and the different domains were later imaged using differential polarization microscopy showing separately the positive and negative bands [18, 20]. While both positive and negative signals prevail in the image averages over the whole membrane (thus including multiple macrodomains), the circular polarization spectrum is heavily influenced by the alignment of the chloroplasts [20,21,22]. Local alignments of the chloroplasts might therefore affect the spatial variation in circular polarization and thus overall the signal on a leaf and canopy scale.

In the present study, we will investigate the spatial components of polarization in vegetation using imaging Mueller matrix polarimetry in transmission in order to get more insight into the polarizing and depolarizing components of vegetation leaves. Various measurements on cultivated maize and maple leaves were taken within the relevant wavelength range (650 nm to 710 nm) of the vegetation absorption band in the red. We show that these measurements improve our understanding of the signals obtained on whole leaves and ultimately aid in interpreting the signals in vegetation remote sensing using circularly polarized light.

2. Materials and methods

2.1. Sample preparation

Maize (*Zea mays*) was grown in the laboratory of Colleen Doherty, Department of Molecular and Structural Biochemistry, North Carolina State University. The wild types we used were N78S and N74R. No differences in their growth features (V3) were observed during the measurements. The plants were cultivated in sand at a 16 h/8 h light-dark regime (at a photon flux density of 600 μmol $m^{-2} s^{-1}$ photosynthetically active radiation (400 nm to 700 nm)) at room temperature. All spectroscopic measurements on the maize leaves were carried out with the leaves still attached to the plant. Maple (*Acer rubrum*) leaves were collected in November from trees growing at the Centennial Campus, North Carolina State University in Raleigh. In order to prevent dehydration, the petioles or stems of the leaves were placed in water after collection and during the measurement.

2.2. Polarization and Mueller matrix decomposition

Polarization in general is often described in terms of the four parameters of the Stokes vector **S**. With the electric field vectors E_x in the x direction (0°) and E_y in the y direction (90°), the Stokes vector is given by:

$$\mathbf{S} = \begin{pmatrix} I \\ Q \\ U \\ V \end{pmatrix} = \begin{pmatrix} \langle E_x E_x^* + E_y E_y^* \rangle \\ \langle E_x E_x^* - E_y E_y^* \rangle \\ \langle E_x E_y^* - E_y E_x^* \rangle \\ i\langle E_x E_y^* - E_y E_x^* \rangle \end{pmatrix} = \begin{pmatrix} I_{0°} + I_{90°} \\ I_{0°} - I_{90°} \\ I_{45°} - I_{-45°} \\ I_{RHC} - I_{LHC} \end{pmatrix}. \quad (1)$$

The Stokes parameters *I*, *Q*, *U* and *V* refer to intensities which thereby relate to measurable quantities. The absolute intensity is given by Stokes *I*. Stokes *Q* and *U* denote the differences in intensity after filtering linear polarization at perpendicular directions, where Q gives