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Measurement and interpretation of Mueller matrices of barley leaves

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Abstract. **We report the results of measuring Mueller matrices for three groups of leaf samples of common barley (*Hordeum vulgare*): *Chlorina* mutant, *Chlorina* etiolated mutant and *Cesaer* varieties. The repeatability of the measurement results of Mueller matrices of such a complex and highly depolarising object as a plant leaf is demonstrated. It is shown that the barley leaves of these three groups can be reliably identified both at forward scattering and backward scattering modes; the best results are obtained in the case of forward scattering. In both cases, the most informative matrix elements are identified. It is also shown that at backward scattering mode linear dichroism comes out, the magnitude of which increases with decreasing observation angle.**

Keywords: *Mueller matrix polarimetry, barley leaf, Mueller matrix, Stokes vector, degree of polarisation, dichroism.*

1. Introduction

Plant leaves are photosynthetic chemical ‘factories’ used by plants to produce energy substances (carbohydrates) that they need to survive. The fundamental chemical compound in this sense is chlorophyll molecules, which are contained within leaves in specialised structures called chloroplasts. Despite the fact that the chemistry of photosynthesis has been well known for a long time [1], the features of the passage of light through the surface and thickness of a leaf due to the complexity of the latter have been scarcely studied and are of increasing interest today. Mueller matrix polarimetry, which is characterised by high information content and an extremely small effect on the studied object, is a very attractive technique for studying the processes of interaction of light with leaves of various plants [2, 3].

The purpose of this work is:

(i) to measure Mueller matrices of leaves of three groups of barley (*Hordeum vulgare*) samples: group (a), *Chlorina* mutant, which was grown under ordinary lighting conditions; group (b), *Chlorina* mutant, whose plants were etiolated (left in the dark) during growth; and group (c), *Cesaer* varieties;

(ii) to demonstrate the reproducibility of the measured Mueller matrices;

(iii) to demonstrate the possibility of identifying the above groups of barley leaves based on their Mueller matrices; and

(iv) to determine the most informative (sensitive) matrix elements in the context of the problem in question.

2. Mueller matrix polarimetry and features of the experiment

The description of light that is convenient for practical applications (especially in the cases of presence of depolarisation), is its representation in the form of a 4 × 1 vector with real elements, i.e. the Stokes vector $\boldsymbol{S} = [S_1\ S_2\ S_3\ S_4]^{\mathrm{T}}$ (the superscript T means transposition) [4, 5]:

$$\begin{aligned} S_1 &= \langle E_x^2\rangle + \langle E_y^2\rangle = I, \\ S_2 &= \langle E_x^2\rangle - \langle E_y^2\rangle = IP\cos(2\theta)\cos(2\varepsilon), \\ S_3 &= 2\langle E_xE_y\cos\chi\rangle = IP\sin(2\theta)\cos(2\varepsilon), \\ S_4 &= 2\langle E_xE_y\sin\chi\rangle = IP\sin(2\varepsilon), \end{aligned} \tag{1}$$

where I is the intensity of light; E_x and E_y are the Cartesian orthogonal components of the electric vector $\boldsymbol{E}$ of a plane electromagnetic wave propagating along the z axis; χ is the phase shift between the components E_x and E_y; $\langle\ \rangle$ stands for time averaging; θ and ε are the azimuth and ellipticity of the light polarisation ellipse, respectively; and P is the degree of polarisation of light, defined as

$$P = \frac{\sqrt{S_2^2 + S_3^2 + S_4^2}}{S_1}. \tag{2}$$

In our experiment, the probe light beam is completely polarised ($P = 1$). After interacting with the objects under study, the degree of polarisation of the output (scattered) light is a function of the azimuth θ^{inp} and the ellipticity $\varepsilon^{\mathrm{inp}}$ of the polarisation ellipse of the probe light. As follows from (1), the first Stokes parameter S_1 has the physical meaning of the total light intensity. The remaining three Stokes parameters describe the prevalence of linear (vertical/horizontal) polarisations oriented at angles of ± 45° and circular (right, left) polarisations, respectively.

The transformation of the Stokes vector of the input light during its linear interaction with the object under study is

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described by a 4 × 4 matrix with real elements, i. e. the Mueller matrix [4, 5]

$$S^{\text{out}} = MS^{\text{inp}}, \quad \text{where } M = \begin{pmatrix} M_{11} & M_{12} & M_{13} & M_{14} \\ M_{21} & M_{22} & M_{23} & M_{24} \\ M_{31} & M_{32} & M_{33} & M_{34} \\ M_{41} & M_{42} & M_{43} & M_{44} \end{pmatrix}. \tag{3}$$

The Mueller matrix fully characterises anisotropic and depolarising properties of the object in question at a given wavelength of the probe light and for given directions of probing and observation. Below in the text, all matrix elements (except for M_{11}) are given in the normalised form: M_{ij}/M_{11}.

To measure the matrix elements, we used a Mueller polarimeter, whose schematic is shown in Fig. 1. The polarimeter includes two main parts: a probing channel, or a generator of states of polarisation of light, and a receiving channel, or an analyser of states of polarization of light. In this case, the probing channel consists of a light source (*1*) with isotropic polarisation (circular polarisation or unpolarised light), an ideal polariser (*2*), a phase plate (*3*) with controlled azimuths of orientation, and an expander of the probe light beam (*4*); element (*5*) is the object under study. The receiving channel is a Stokes polarimeter and consists of a phase plate (*6*) and an ideal polariser (*7*) with controlled azimuths of orientation, as well as a photodetector (*8*). The distinctive features of calibration and measurements using this polarimeter are considered in detail in [6].

The probe wavelength was 0.632 μm. The probe beam was broadened to 20 mm. This was done to eliminate the influence of local individual characteristics of barley leaves on the measurement results, as well as possible thermal effects on the samples in question. The latter is confirmed by calculated estimates of illumination and experimentally (see Section 4). The probing of the samples during the measurement of Mueller matrices was performed normally on the axial surface of the leaf, with the leaf being oriented as shown in Fig. 1.

The error in determining the matrix elements was estimated experimentally during the polarimeter calibration. To this end, we compared the measured elements of the Mueller matrices with their tabular values in the so-called objectless measurement mode. This is due to the fact that the Mueller matrix, measured in this mode, is a single diagonal matrix, known with the greatest possible accuracy. In addition, we measured other objects whose matrices are known with high accuracy: industrially manufactured prism polarisers and wave plates [4, 5]. The measurement error of the Mueller matrix δM was estimated by the formula:

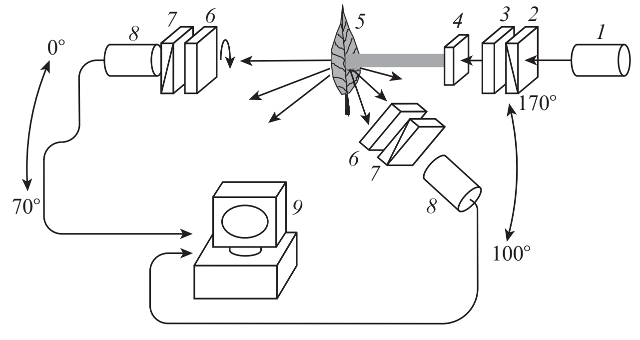

Figure 1. Schematic of a Mueller polarimeter used in the experiment.

$$\delta M = \frac{\lVert M_{\text{theor}} - M_{\text{exp}} \rVert}{\lVert M_{\text{theor}} \rVert}, \tag{4}$$

where M_{theor} and M_{exp} are the exactly known and measured (normalised to M_{11}) Mueller matrices of the object in question; and the metric norm [7] is

$$\lVert M \rVert = \left(\sum_{i,j}^{4} \lvert M_{ij} \rvert^2 \right)^{1/2}. \tag{5}$$

Errors in our experiment did not exceed 2%.

3. Objects of study and their preparation for the experiment

The illumination conditions for etiolating the plants of mutant barley (*Hordeum vulgare*) corresponded to those described in [8]. The plants were grown for 14 days at 25 °C with a photosynthetic photon flux density of 200 μmol m^{-2} s^{-1} and a photoperiod of 16 hours. Etiolated seedlings of *Hordeum vulgare* were grown at 25 °C in the dark for 7 days, after which they were subjected to intermittent illumination for 36 cycles (2 min of illumination at a flow of 40 μmol m^{-2} s^{-1} and 118 min of darkness).

These illumination conditions limit the formation of starch molecules in the leaf and produce large arrays of photosynthetic stacks of thylakoids. Additional light treatment using tungsten halogen lamps (1000 μmol m^{-2} s^{-1}) was performed on separate leaf samples placed on wet filter paper. The leaves were protected from IR radiation by a layer of water.

4. Results and discussion

Figure 2 presents the results of measuring the Mueller matrices for the leaves of three groups of barley in the forward scattering mode in the range of observation angles from 0 to 70°. Figure 3 shows the results of measurements of the Mueller matrices in the backward scattering mode in the angle range of 100° – 170°. For observation angles in the range from 70° to 100°, the intensity of the scattered light is close to zero. Each point in Figs 2 and 3 corresponds to two averaging processes: over 300 single measurements for one sample and over 10 samples of the same group. The exposure time when measuring one sample is 20 min.

It follows from Fig. 2 that the Mueller matrices for all three groups of barley in the forward scattering mode have the form

$$M^{\text{f}} = \begin{pmatrix} M_{11} & 0 & 0 & 0 \\ 0 & M_{22} & 0 & 0 \\ 0 & 0 & M_{33} & M_{34} \\ 0 & 0 & M_{43} & M_{44} \end{pmatrix}. \tag{6}$$

Note that for barley samples of groups (b) and (c), the form of matrix (6) is characteristic in the entire range of observation angles from 0 to 70°, while for samples of group (a) the matrix elements M_{34} and M_{43} are nonzero only in the range of angles from 45° to 70°.

In the case of backward scattering in the range of angles from 100° to 170°, the Mueller matrices for barley samples of groups (a) and (b) (see Fig. 3) have the form

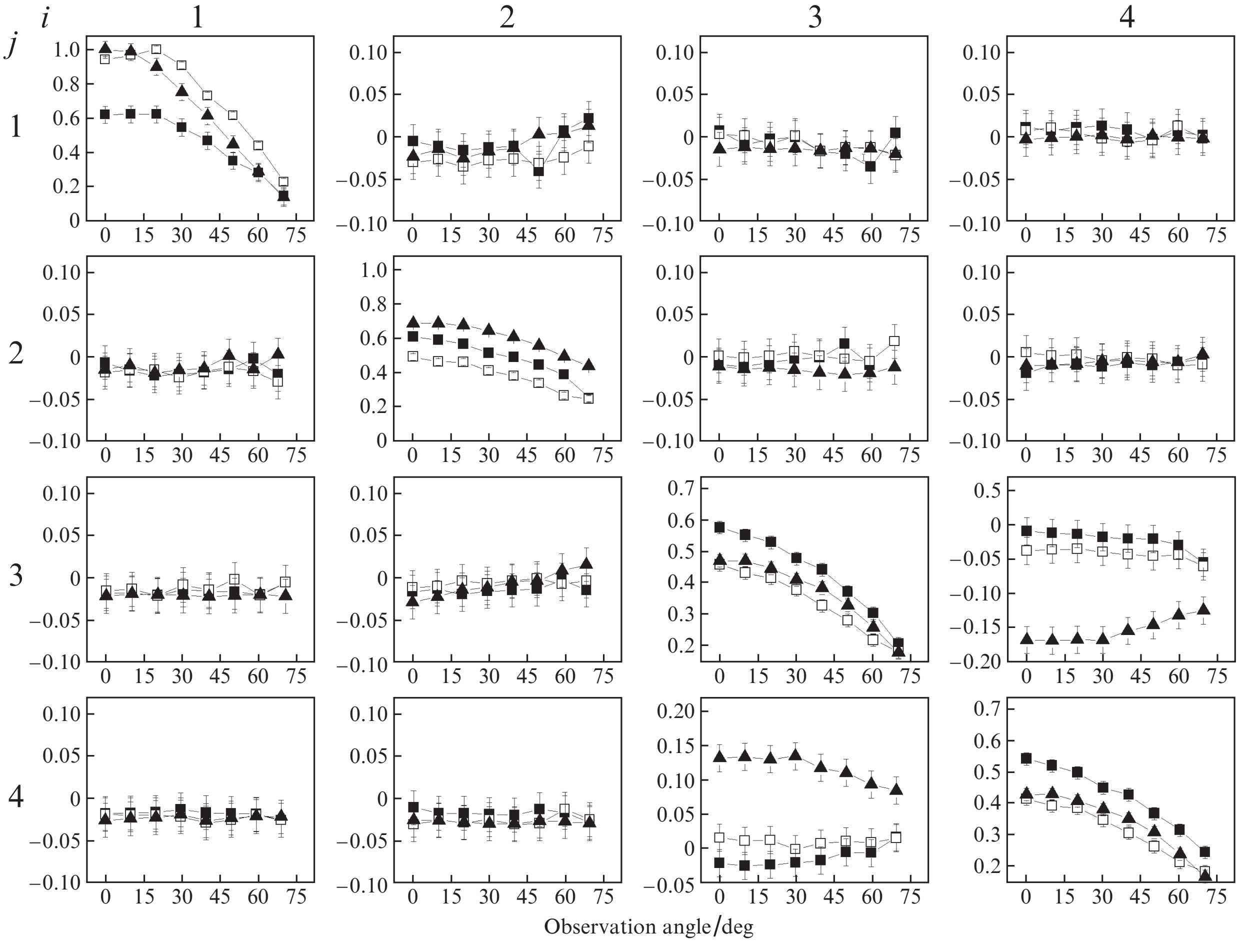

Figure 2. Matrix elements M_{ij} for leaves of three groups of barley as functions of the observation angles in the forward scattering mode [■, group (a); □, group (b); and ▲, group (c)].

$$M^{\mathrm{b}} = \begin{pmatrix} M_{11} & M_{12} & 0 & 0 \\ M_{21} & M_{22} & M_{23} & 0 \\ 0 & M_{32} & M_{33} & M_{34} \\ 0 & 0 & M_{43} & M_{44} \end{pmatrix}. \qquad (7)$$

For the samples of group (c), the values of the matrix elements M_{23} and M_{32} in this case are close to zero in the entire range of observation angles.

Taking into account that the wavelength of the probe light was chosen based on the spectral features of light absorption by chlorophyll molecules [groups (a) and (b)] [9], comparison of Figs 2 and 3 points to the fact that the matrix elements M_{12} and M_{21} for forward scattering are close to zero, and for backward scattering they are nonzero in the entire range of observation angles. The fact is that it is customary to associate nonzero values of these elements with the presence of anisotropic absorption both in the framework of the approximation of continuous [4, 5] and discrete [10, 11] media. It can be seen that in the backward scattering mode, the degree of linear polarisation under probing with unpolarised light substantially depends on the observation angle [10–12]; the effect is maximum for samples of groups (a) and (c), while for samples of group (b) it is relatively small.

At the same time, the equality of the elements M_{12} and M_{21} to zero in the forward scattering mode cannot apparently be unambiguously associated with the absence of anisotropic absorption. A more realistic interpretation is that in the forward scattering mode the absorption anisotropy is small and its presence is ‘masked’ by a high degree of depolarisation of the scattered light. The observed high degree of scattered light depolarisation and its increase with increasing observation angle are explained, obviously, by an increase in the multiplicity of light scattering [13–15]. This is confirmed by the character of the dependence of the element M_{22} on the observation angle, the difference of which from unity is interpreted as the degree of depolarisation of the input linearly polarised light [10, 11]. Note that in the forward scattering mode, the value of the matrix element M_{22} substantially depends on the observation angle, while in the backward scattering mode the dependence is virtually absent. Moreover, the matrix element M_{22} is the most sensitive for the identification of the studied groups of barley leaf samples in the direct scattering mode. In the backward scattering mode, the sensitivity of M_{22} is noticeably lower, and the distinction of all three groups of samples is possible only in the range of observation angles from 130° to 170°.

The nonzero values of the matrix elements M_{34}, M_{43}, M_{23} and M_{32} apparently suggest that there is a noticeable change in the phase between the components of the electric vector $\boldsymbol{E}$ of electromagnetic radiation as a function of the length of the optical path travelled in the leaf volume. This is observed in samples of group (c). Samples of group (b) also demonstrate

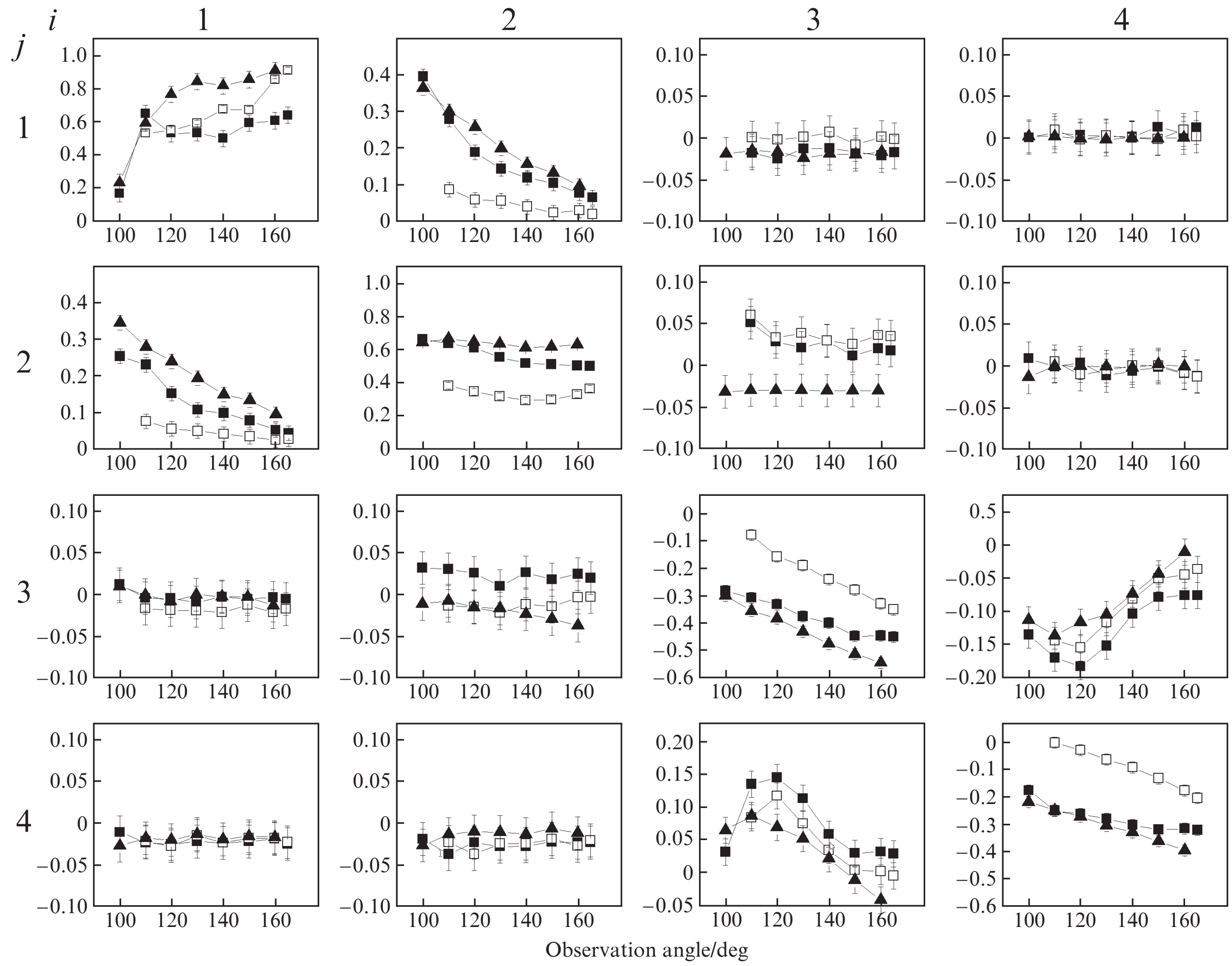

Figure 3. Matrix elements M_{ij} for leaves of three groups of barley as functions of the observation angles in the backward scattering mode [■, group (a); □, group (b); and ▲, group (c)].

this property, but to a lesser extent. This allows us to conclude that the state of polarisation of light plays a significant role in the interaction of light with a leaf.

Taking into account the value of $1 - M_{22}$, which is a measure of the nonsphericity of scattering particles [10, 11], and the difference between the matrix element M_{33} and M_{44} for transmitted light, the results shown in Fig. 2 allow us to conclude [12] that in the case of forward scattering the nonspherical (elongated) particles or structures in the leaf play a decisive role. For the backscattering angles (see Fig. 3), the values of the elements M_{33} and M_{44} are approximately the same, which indicates the prevailing influence of spherical particles on the character of backward scattering of light.

One of the goals of this study was to determine the reproducibility of the obtained experimental results. To this end, we measured the elements of the Mueller matrix for ten samples of barley leaves of group (c) with the orientation of the samples shown in Fig. 1. The measurements were performed for an observation angle of 0°. Then, the matrix elements were measured for the same samples at different orientations with respect to the z axis in the range of angles from 0° to 180° with a step of 10°. After that, the Mueller matrices for the initial orientation of all samples were numerically rotated to 180° with a step of 10° [7]. The results obtained for matrix elements for all rotation steps, averaged by the two above methods, are presented in Fig. 4.

One can see from Fig. 4 that for some rotation angles, the deviations of the results slightly exceed 2%. This can be explained by the fact that in practice there are additional errors in the orientation of the samples, which, obviously, are absent in the numerical calculation. Nevertheless, there is a fairly good agreement between numerical and experimental data.

Note that during measurements the leaf samples were rotated for 2 hours. This was done to experimentally confirm that the thermal effect on the leaf is absent during the measurements. Otherwise, during the experiment, we would obviously observe an increase in the difference between the experimental and numerical results.

5. Conclusions

We have presented the results of measuring Mueller matrices for three different groups of barley leaves and demonstrated the repeatability of the measurement results. The barley groups selected for the study differ in the internal structure of the leaf, which is achieved either due to mutation or by illumination during the growth. These differences determine the observed features of the polarisation characteristics of the studied samples, shown in Figs 2 and 3. In this regard, of particular interest is the question of whether barley leaf groups can be identified on the basis of their Mueller matrices. An analy-

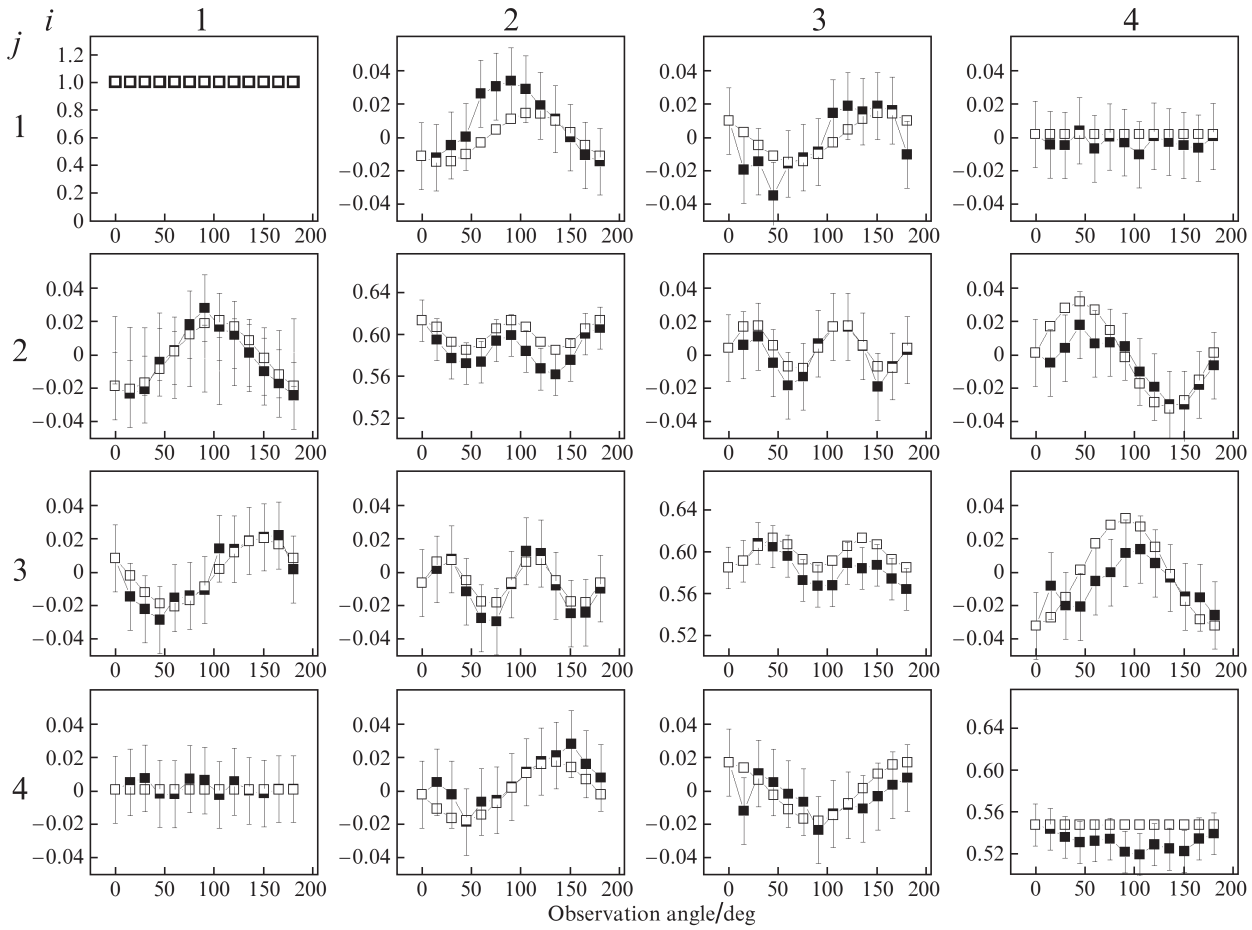

Figure 4. Reproducibility of the measurement results of matrix elements M_{ij} for barley leaves (■, experiment; □, numerical calculation).

sis of the results obtained in this paper gives, in our opinion, an unambiguously positive answer to this question.

Moreover, the three studied groups of barley leaves can be identified in two experimental geometries, i. e. in forward or backward scattering modes. At the same time, as follows from a comparison of the results, forward scattering is the best option. The matrix elements M_{22}, M_{34} and M_{43} are the most informative in this context, while the matrix elements M_{33} and M_{44} are somewhat less sensitive. In the backward scattering mode, the diagonal elements M_{22}, M_{33}, M_{44} and the elements M_{12} and M_{21} are the most informative. Nonzero values of the elements M_{12} and M_{21} indicate that at backward scattering mode linear dichroism comes out, the magnitude of which increases with decreasing observation angle.

Thus, the nature of the change in the elements of the Mueller matrices on the observation angle (see Figs 2 and 3) demonstrates the dependence of the absorption and scattering of light by barley leaves on the polarisation state of the probe light. This is important for understanding the process of penetration of light into the leaf and the evolutionary adaptation of the internal structures of the leaf to the absorption of light.

It must be emphasised that the interpretation of the Mueller matrices in our experiment was carried out in the context of the so-called direct polarimetry problem. In other words, we talked about the parameters of some effective object model within the framework of the discrete medium approximation, which is described by Mueller matrices similar to (6) and (7) for forward and backward scattering modes. For a quantitative subject analysis of information about the depolarising and anisotropic properties of the studied barley leaf samples, we plan to analyse the experimental Mueller matrices presented in this paper based on existing methods for decomposing Mueller matrices [16–18] and depolarization metrics [19–21]. In addition, to clarify issues related to the mechanisms of depolarisation of the probe light, including the contribution of chlorophyll fluorescence to the observed depolarisation of the probe light, it may be necessary to conduct additional experiments to measure Mueller matrices at other wavelengths and/or using polychromatic (nonpolarised) probe light.

It is interesting to note that Mueller matrices (6) and (7) differ from the Mueller matrices for oak leaves depending on the moisture content (see (10) and (11) in [22]) and wheat leaves for different growth conditions and infection levels (see Figs 5 and 6 in [23]) measured in the same experimental geometry. This fact and the results of the analysis of the Mueller matrices presented in this work allow us to state that various types and conditions of plants can also be reliably distinguished using Mueller matrix polarimetry. The latter, in turn, can be used to solve a number of important practical problems, for example, to assess the quality of the crop under certain weather conditions or to diagnose plant diseases.

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