Spectrometric method for measuring light absorption by plant leaves

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Abstract. This article describes the development of the experimental setup for researching the plant leaves absorption spectrum and its biological testing. The spectrometric method based on measurement of tissue optical properties by means of double-integrating-sphere system. During the study of light absorption by lettuce leaf it was noted that the total absorption of living and freshly cut leaf is identical, but changes over time after the cut. The "absorption map" of the lettuce leaf surface was compiled.

1. Introduction
Light plays one of the key roles in plant life. Plants use the reaction of photosynthesis to convert light into chemical energy and produce organic molecules.

A number of methods have been developed and a large number of parameters describing the nature of biochemical and morphological reactions have been identified to assess the influence of lighting parameters on plant growth and development. The most common include leaf morphology, the structure of chloroplasts, content of pigments, diffusion of carbon dioxide, the efficiency of photosynthesis [1]. Genetic factors play a separate role. For example, in [2] to understand the inheritance mechanism of plant response to light depending on the habitat, intravidual variations of light-dependent processes of seedling development were characterized. Three photoreceptor systems absorbing red/far red, blue/near ultraviolet, ultraviolet light and subsequently interact with other elements of signal transduction were identified, it is ultimately leads to molecular and morphological responses [3].

However, genetic analysis is quite complex in execution, besides, the method is destructive and does not take into account the influence of external factors. Therefore, in addition to genetic predisposition, much attention is paid to the optics of the plant leaves. Changes in the spectral characteristics of the transmitted light, intensity and maximum wavelengths can be a sensitive indicator of the physiological state of the plants and characterize its needs, especially in relation to photosynthetic photon flux.

The transformation of light occurs in the plant leaves by using the photosensitive pigments, the main of them are chlorophyll a and b. Due to the presence of additional pigments (carotenoids, anthocyanins, etc.) in the cells, the area of absorption of the visible radiation range expands. The light
absorption by plant leaves differs from the absorption of pigments extracted from them and measured in the solution. The optical parameters of leaves not only characterize the amount of light energy absorbed and processed by the plant, but can also be associated with processes such as pigment degradation and other biomolecular changes [4]. Also, the broadening of the absorption spectrum of leaves is associated with the Mie scattering in structures such as mitochondria, ribosomes, nuclei, starch grains and other plastids [4]. Currently, overall spectral libraries [5] are being created and methods of leaf absorption diagnostics are being developed [6] Leaf absorbance spectra convey information about the structure and biological constituents inside plant tissues and can be directly linked to the physiological processes of plants, and thus, to biomass production, harvest images, and health of plants [6]. The aim of this work was to develop a non-destructive method for registering absorption spectra of plant leaves, potentially associated with the physiological condition of plants and photosynthetic apparatus and correlated with growth conditions – lighting, humidity, temperature, etc.

2. Experimental spectrometric method
The spectrometric method based on measurement of optical properties of tissue by using the double-integrating-sphere system was taken as a basis in the development of the design of the experimental setup [7] – the studied sample is placed between the spheres, one of which (IS1) registers reflected light, and other (IS2) transmitted light (Fig. 1).

Integrating spheres with the internal diameter equal 50 mm were designed in SPbPU and were made on a 3D printer from ABS plastic. Coloring of the inner surface of the spheres was made in accordance with appendix D GOST R 55702-2013, the optical properties were tested in comparison with the reference surface of the sphere EVERFINE. The spectral reflection coefficient of the color was $85\pm5\%$. As a source of radiation in the scheme were used specialized under photosynthetically active range led module developed in the Russian engineering company "O2 Lighting Systems". It contains five channels with maxima in the wavelength range: 650-670 nm, 720-740 nm, 440-460 nm, 415-435 nm and 4500K white light, and driver-controlled intensity of the radiant flux of each channel from 0 to 255 arb.unit, which allows to study the effect of the desired region of the spectrum and the radiation energy on the object. The light from the source was collected using a converging lens and then through the collimator 74-UV (Ocean optics) with a beam divergence $< 2^\circ$ was brought into the integrating sphere IS1 falling on the sample area with a diameter of 10 mm. As a detector it was taken optical fiber CCD spectrometer CCS200 (Thorlabs) with a spectrum range from 200 to 1000 nm with wavelength division $<2$ nm, coupled with integrating spheres by the fiber BFL200HS02 (Thorlabs)
with a diameter of 200 µm. The absorption coefficient $A(\lambda)$ was calculated with the formula $A(\lambda) = 1 - R(\lambda) - T(\lambda)$, where $R(\lambda)$ is the reflection coefficient of the sample, $T(\lambda)$ is the transmittance.

3. Biological testing and discussion of results

Biological testing of the developed experimental setup for measurement of light absorption by plant leaves was carried out. As the object of study was selected lettuce "Aficion".

The proposed method is not damaging and allows to fix the absorption spectra of plants in the process of their normal development, without affecting the object under study. However, in some cases it may not be possible to register the spectrum directly from the live plant. To determine the time during which the difference between the living and the cut leaf will not be recorded was carried out an experiment. The first was registered the absorption spectrum of lettuce growing in the ground and not separated from the rest of the bush. Then without changing the position the plant leaf was separated from the main part of the plant and absorption spectrum was immediately measured. After that two more spectra were recorded after 1000 and 5000 seconds past cut. The results of the experiment are shown in figure 2. Differences in the absorption spectra of living and the freshly cut leaves are not observed, but after a while the absorption of the cut leaf changes. After 15 minutes, the absorption spectrum of the cut leaf decreases in intensity over time. If the cut leaf is additionally irradiated (for example, from a source in the measuring circuit for a long time), its absorption, on the contrary, increases. An hour after the cut off, not only the absorption intensity changes, but also the form of the absorption spectrum. Intensity in blue range of the spectrum from 400 to 500 nm and red region from 665 to 685 nm increase. There is also a decrease in the absorption intensity in the region from 630 to 660 nm compared to the absorption by living leaf. It is interesting to note that the proportion of light absorbed by the leaf nearby 550 nm is practically unchanged over time. Thus, the absorption spectrum of the living and freshly cut leaf within 5 minutes does not differ from each other, which indicates the possibility of measurements on freshly cut leaves in the case when it is not possible to place the experimental setup directly next to the object under study.

![Figure 2. Absorption spectra of the lettuce leaf surface with a diameter of 10 mm. 1 – living leaf, 2 - freshly cut leaf, 3 - 1000 s after the cut, 4 - 5000 s after the cut.](image)

Also the "absorption map" of the lettuce leaf surface from the top to the base was compiled. Map of the leaf absorption was registered over the whole area of the leaf in accordance with the limits of 10 mm diameter each, schematically indicated in figure 3A. Total absorption depends on the part of the leaf and differs across the surface of the leaf in the range from 30 to 50 % (Fig. 3b). The highest values are typical for the edges of the leaf, and there is a certain symmetry in the absorption. The absorption coefficient in the areas where the stomata located are less, the smallest value is typical for the base, that is, for the oldest part of the leaf. The values of total absorption in the blue region (400-
500 nm) varied between 14-21% of the total, in the green region (500-600 nm) 7-13% of the total, in the red region 9-16% of the total. Moreover, smaller values are typical for the base of the leaf.

Figure 3. "Absorption map" of lettuce: the scheme of leaf registration areas (a), the integral absorption coefficient for each leaf area (b).

4. Conclusion
When growing plants in closed ground conditions or in greenhouse complexes with a lack or absence of natural light, the key is the correctly selected spectrum, intensity and duration of artificially created lighting. One of the variants of phytomonitoring can be the analysis of the absorption spectrum of the plant leaf. The used spectrometric method will provide information about the absorption of radiation from the lighting source and associated photosynthetic reactions. Changes in the optical properties of leaves can be a sensitive indicator of the physiological state of the plant and characterize its needs, especially in relation to photosynthetically active radiation.

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