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Spectral Evaluation of the Vital State of *Quercus Robur* L. Under Simulated Drought Conditions

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Abstract

Non-destructive spectral methods of analysis are increasingly being used to study the content of plant metabolites, evaluate morpho-physiological and biochemical indicators, as well as evaluation of the vital state. Visualization of the vital state through spectral profiles can provide a more detailed picture of plant adaptation to stress. To model experimental drought, 5-6 month-old *Quercus robur* L. seedlings were divided into three groups: control and experimental groups with and without watering (drought), with 15 seedlings in each group. Spectral evaluation of leaf blades was performed using a portable spectroradiometer SpectraPen SP110 Uvis and a plant analyzer Dualex Scientific+ at 0 (control), 168 (one week), and 336 (two weeks) hours. As a result of spectral analysis, spectrograms of radiation absorption of *Q. robur* leaf blades were obtained, as well as the content of the sum of chlorophylls, flavonols and anthocyanins under watering and drought conditions. The study revealed changes in the spectrograms of absorption of *Q. robur* leaves related to the content of metabolites. The difference in absorption peaks between the groups became more expressed over time under the influence of drought. The pigment content in the leaf blades varied during the experiment, which indicates plant adaptation to stress. Preliminary results of the study can be used to expand knowledge about ways to evaluate the vital state of woody plants in the field.

Keywords: spectral analysis, leaf blade, pigments, *Quercus robur* L., drought.

1. Introduction

Non-destructive methods of analysis are increasingly being used to study the vital state of plants and the content of various metabolites [1-3]. These methods allow for the measurement of substance content without damaging or destroying plant organs, which is particularly important when multiple measurements are required. Spectral methods enable the evaluation of various morpho-physiological and biochemical indicators of plants, as well as their vital state, without the need for time-consuming sample preparation in both field and laboratory conditions in response to various stress factors. [4-7].

However, the main issue lies in the frequent evaluation of only well-known metabolites, without taking into account those that can be detected at other wavelengths.

Additionally, the analysis often focuses on the content of entire groups of substances, such as flavonoids [8], carotenoids [9], etc. Therefore, visualization of the vital state through spectral profiles can provide a more comprehensive picture of plant adaptation to stress factors.

The object selected for the pilot study of spectral profiles was the pedunculate oak (*Quercus robur* L.), known for its high drought resistance and being one of the main woody species used for agroforestry and protective afforestation in southern Russia [10, 11]. Therefore, conducting a spectral analysis of the leaf blade can provide a comprehensive understanding of how *Q. robur* responds to drought.

The aim of this study was to conduct a pilot study to evaluate the spectral profiles of *Q. robur* leaf blades under simulated drought conditions.

2. Materials and methods

To model experimental drought, 5-6 month-old *Q. robur* seedlings without signs of phytopathology were used. The seedlings were divided into three groups: a control group, a group with watering and a group without watering (drought), with 15 seedlings in each group. Spectral diagnostics of *Q. robur* leaves was performed at 0 (control), 168 (one week) and 336 (two weeks) hours. After measuring the spectral parameters of the leaves of the control group, the temperature in the experimental room was set to 30 °C.

A portable spectroradiometer SpectroPen SP110 Uvis (Photon Systems Instruments, Czech Republic) [12], which registers radiation in the wavelength range from 340 nm to 790 nm, was used to obtain spectral profiles of the leaf blades of Q. *robur* seedlings under study. Optical radiation was recorded from the abaxial side of the leaf blade next to the midrib. The reference radiation was sunlight, with the optical path passing through the registration site on the leaf blade. A specialized program SpectraPen v.1.1.0.10 (Photon Systems Instruments, Czech Republic) was used to export the primary spectral profile data of *Q. robur* leaf blades. The obtained data in the .scv format were converted to Excel spreadsheet format (Microsoft, USA) and used for further statistical processing.

The content of the sum of chlorophylls, flavonols, and anthocyanins in μg/cm^2) in the leaves epidermis was measured using a plant analyzer Dualex Scientific+ ("Force-A", France).

The formula below was used to construct spectrograms of radiation absorption:

$$A = log\frac{I_0}{I},$$

where A is the optical density coefficient, I_0 is the reference radiation intensity, I is the measured radiation intensity.

Quantitative data were analyzed using Statistica 12.0 software (StatSoft Inc., USA) [13]. The indicators used to evaluate nonparametric samples in biological studies were calculated: normality of value distribution, median [1st quartile, 3rd quartile], and the significance of sample differences was analyzed. To determine the differences between two independent samples, the Mann–Whitney test was used at a significance level of $p<0.05$.

3. Results and discussion

As a result of the study, spectrograms of radiation absorption of *Q. robur* leaf blades were obtained in the wavelength range of 340-790 nm under watering and drought conditions at three time intervals: 0 (control), 168 (one week), and 336 (two weeks) hours. The spectrograms of radiation absorption had two peaks: a higher and narrower peak in the range of 415-480 nm, as well as a lower and broader peak in the range of 481-635 nm (Fig. 1). The absorption peaks were highest in the control group and decreased in the experimental groups. Moreover, the most significant differences in absorption values were observed in the wavelength ranges corresponding to the aforementioned peaks, while differences outside these ranges were minimal. This indicates that drought stress affected the leaves ability to absorb light. At the same time, the leaf blades changed over time under the influence of stress factors (Fig. 2A). In this case, spectrograms help to track changes in the vital state of *Q. robur* leaf blades.

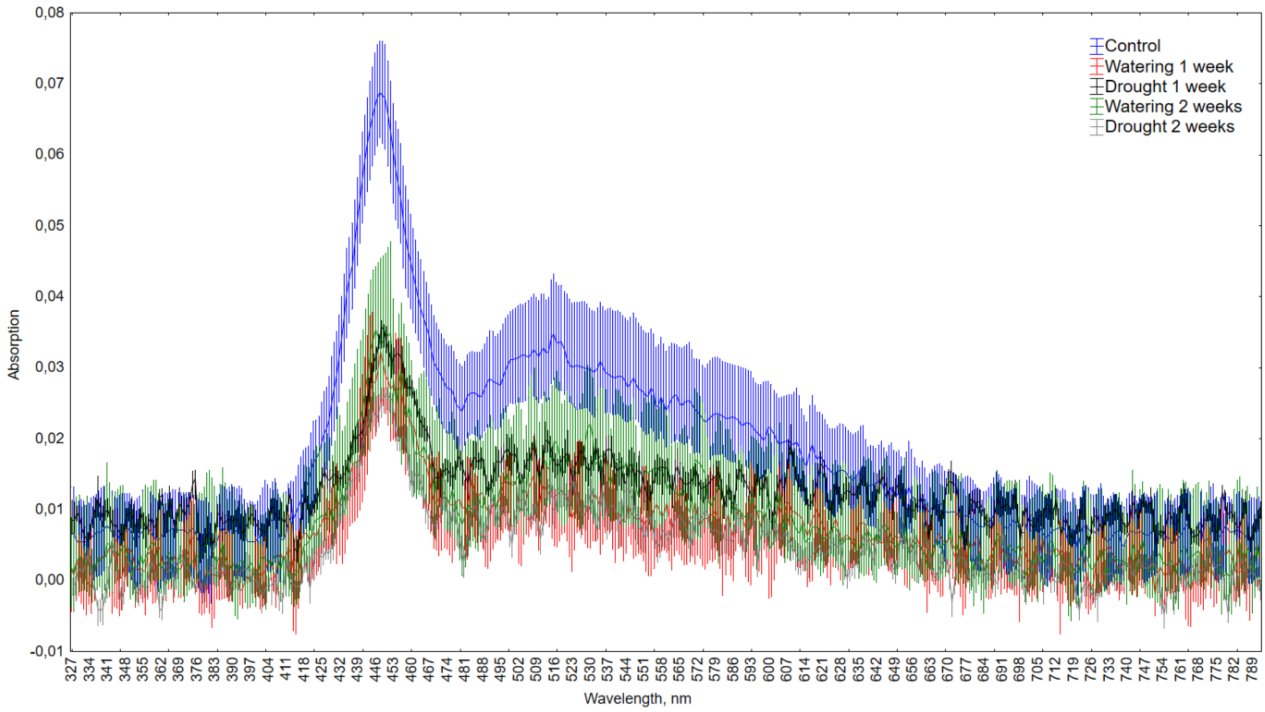


Fig. 1. Spectrograms of radiation absorption of *Q. robur* leaf blades under simulated drought conditions

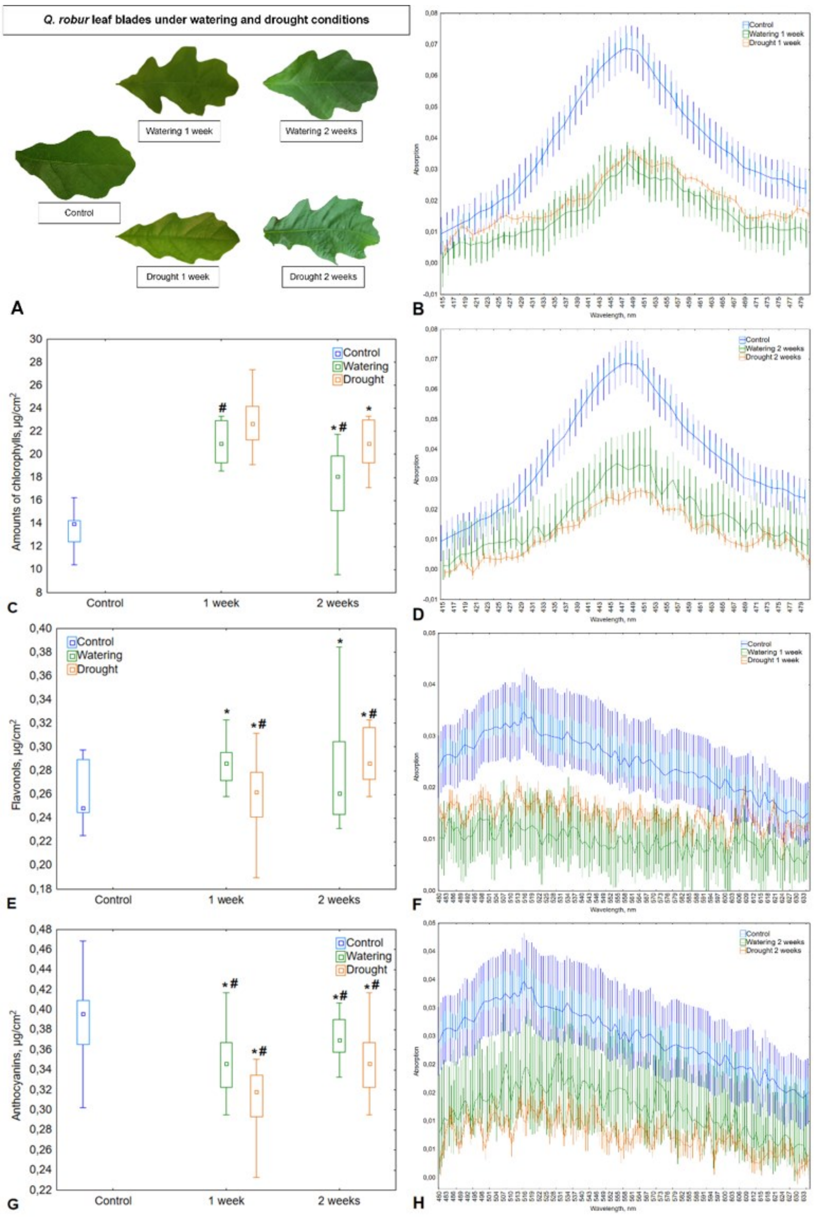
Based on the literature data, it can be assumed that the first peak represents the peak of chlorophylls and carotenoids [14-16]. The second broad peak can be formed by anthocyanins [17, 18]. The peak of flavonols is not observed. This may be due to the fact that the device does not record values at all wavelengths for flavonols [19], as well as the possibility that the peak for flavonols is small and may be overlapped by other metabolites. Subsequently, to study the vital state of the leaf blade (Fig. 2A) and its connection to the spectral profile of radiation absorption (Fig. 2B, 2D, 2F, 2H), spectra were taken at wavelengths of 415-480 nm and 481-635 nm.

The *Q. robur* leaf blades were visually evaluated. One and two weeks after the beginning of the experiment, it was observed that the leaf blades from the watering group did not differ much from the control group. At the same time, leaf blades from the drought group showed precise signs of wilting.

Figures 2B and 2G show a significant difference in absorption peaks (2.5-3 times) between the control and experimental groups in the 415-480 nm range. Two weeks after the beginning of the experiment, the spectral values indicate a more pronounced difference in absorption (1.5 times) between the watering and drought groups.

Figures 2F and 2H also demonstrate significant differences between the groups in the 481-635 nm range. Compared to the control peak, the absorption is 2-3 times lower in the experimental groups. In the graphs of the first week, absorption is slightly higher in the experimental drought group than in the watering group. However, this situation changes after two weeks.

Statistically insignificant differences are observed in the watering and drought graphs in the first week at the following wavelengths: 416, 435, 436, 439-445, 452, and 470 nm. Additionally, the differences between the control and drought groups in the second week are not significant at wavelengths 434-435, 472-473, and 476-477 nm. In Figures 2E and 2H, no statistically significant differences are observed between drought and watering in the first week at wavelengths 517 nm and 593 nm, and between control and drought at 607, 608 nm. In the second week, $p>0.05$ between watering and drought groups at wavelengths 483-485, 504, 513-514, 544, 587, 592-593, 597, 602, 605-606, 615, 619, 621-622, 624, 627-628, and 634 nm.



Notes: * - statistically significant differences between different experimental groups within the same time intervals (p<0.05); # - statistically significant differences between the same experimental groups at different time intervals (p<0.05)

Fig. 2. Vital state, pigment content and the spectrograms of radiation absorption of *Q. robur* leaf blades under simulated drought conditions: A – the vital state of *Q. robur* leaf blades; B – the first peak of the spectrograms of radiation absorption after one week of the experiment; C – comparison of the sum of chlorophylls content in leaf blades; D – the first peak of the spectrograms of radiation absorption after two weeks of the experiment; E – comparison of the flavonol content in leaf blades; F – the second peak of the spectrograms of radiation absorption after one week of the experiment; G – comparison of anthocyanin content in leaf blades; H – the second peak of the spectrograms of radiation absorption after two weeks of the experiment

Figures 2C, 2E, and 2G present diagrams of the sum chlorophylls, flavonol, and anthocyanin content, respectively. In the control group, the chlorophyll content was 14 μg/cm². After one week, the chlorophyll content increased by 1.5-2 times in the experimental groups, with the drought-simulated group showing higher chlorophyll levels compared to the watering group. After another week, the chlorophyll content in the experimental groups slightly decreased compared to the previous week (18 μg/cm² and 21 μg/cm² for the watering and drought groups, respectively). The chlorophyll level in the drought group was slightly higher than in the watering group. It is hypothesized that plants began to produce more chlorophyll in response to stress. One possible reason for this increase may be a stress-induced response that leads to an increase in chlorophyll production for a certain period of time, which allows maintaining photosynthesis levels and ensuring survival [20, 21].

It is known that the content of flavonols can vary under different stress conditions [22], including an increase under drought stress [23-25]. Compared to the control, the content of flavonols in the experimental groups slightly increased ($p < 0.05$). The content of anthocyanins decreased by approximately 1.5-2 times, with the drought group showing lower anthocyanin content compared to the watering group. After two weeks, the content of anthocyanins increased compared to the first week. Presumably, this is associated with the plant adaptation to stress, in this case, an increase in the level of anthocyanins.

Statistically significant differences in the sum of chlorophylls were found between the first and second weeks of watering, as well as between the second week of watering and the second week of drought ($p < 0.05$) (Fig. 2B). The difference in flavonol content was significant for all groups ($p < 0.05$), except for values between the watering groups in the first and second weeks (Fig. 2D). In the graph showing anthocyanin content, values between different drought and watering groups are statistically significant ($p < 0.05$). Statistically insignificant differences from the control group were found only between the flavonol content in the first week of drought and the second week of watering.

Thus, the changes in pigment content caused by the adaptation of *Q. robur* seedlings to stress led to alterations in the spectrograms of the corresponding pigments. However, due to the use of an uncontrolled radiation source, the obtained spectrograms may differ from the reference spectrograms of the corresponding pigments acquired using invasive methods [26]. According to the Bouguer–Lambert–Beer law, the obtained optical density spectrograms represent the sum of radiation absorption not only by the pigments but also by other metabolites and chemicals present in the leaf blade, as well as a result of scatter of solar radiation by the plant tissue of the leaf blade. The absorption spectrum of chlorophyll and other substances can be affected by environmental factors, such as pH, temperature, the presence of other molecules, and changes in chlorophyll structure due to stress [27, 28]. These factors can cause the absorption peak to shift towards the blue region and decrease in the red region.

During the simulation of experimental drought in *Q. robur*, changes in the absorption spectrograms of leaf blades and the quantitative content of pigments were recorded, which may be related to the impact of water deficiency on the physiological processes of plants. The observed differences in the spectrograms indicate changes in the content of pigments and other metabolites, as well as structural alterations in the leaf caused by drought.

It is hypothesized that the first peak in the spectrogram corresponds to the peak of chlorophylls and carotenoids, while the appearance of the second broad peak indicates the presence of anthocyanins, and the peak for flavonols was not observed. Differences in absorption peaks were observed between control and experimental groups in the wavelength ranges of 415-480 nm and 481-635 nm. In the first week, absorption was slightly higher in the drought group compared to the watering group. Two weeks after the experiment commenced, the differences in absorption between the groups became more pronounced: compared to the control, the peak in the experimental groups was 2-3 times lower.

It is known that plants begin to produce more chlorophyll and other substances to enhance photosynthesis efficiency and ensure survival under stress conditions, such as drought. In the experimental groups, an increase in the chlorophyll content was observed. In the control group, the chlorophyll content was 14 μg/cm^2. After one week, the chlorophyll content increased by 1.5-2 times, and after another week it slightly decreased (18 μg/cm^2 in the watering group and 21 μg/cm^2 in the drought group). In the experimental groups, there is an insignificant increase in the flavonol content compared to the control group. In most cases, the values of absorption peaks have statistically significant differences ($p<0.05$). At the same time, the anthocyanin content decreased by approximately 1.5-2 times ($p<0.05$) in all experimental groups compared to the control. The lowest anthocyanin content was observed in the drought group compared to the watering group after one week of the experiment. However, in the second week, the anthocyanin content increased, which may indicate plant adaptation to stress and the recovery of the anthocyanin production level.

4.Conclusion

During the pilot study, it was revealed that changes in the spectrograms of radiation absorption of *Q. robur* leaf blades are associated with possible alterations in the content of a wide range of metabolites such as chlorophyll, carotenoids and anthocyanins. It was found out that the difference in absorption peaks between the control and experimental groups became more pronounced over time, indicating the impact of drought conditions. In the control group, the spectrograms of radiation absorption demonstrated the highest ability to absorb radiation by leaves, while in the experimental drought groups it decreased. Simultaneously, *Q. robur* leaf blades underwent morphological changes under stress conditions.

It can also be concluded from the results of the study that exposure to stress factors, such as drought, contributes to an increase in the plant chlorophyll content, enhancing photosynthesis efficiency and contributing to their survival. The flavonol content slightly increases, which may be due to the absence of phytopathologies. At the same time, the anthocyanin content decreases under stress conditions. However, their recovery occurs over time, indicating plant adaptation to stress conditions.

Thus, the spectrograms allow tracking changes in the viability of *Q. robur* leaf blades, most noticeable in the range corresponding to the absorption peaks on the obtained spectrograms. Further investigations are needed to study spectral profiles and their connection to plant adaptation in response to stress factors, particularly, drought. The obtained preliminary results can be used in the future to develop methods for evaluating vital state, physiological and biochemical parameters and calculated indexes of woody plants in the field.

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