ADVANCING PLANT METABOLISM ANALYSIS: A REAL – TIME OPTICAL APPROACH, INSIGHTS FROM *VRIESEA CARINATA WAWRA*

Sara V. Ristić[1], Miloš S. Mošić[1], Marija M. Petković Benazzouz[1], Stefan Lekić[2], Katarina M. Miletić[1]

[1]University of Belgrade, Faculty of Physics, Studentski trg 12-16, 11001 Belgrade, Serbia, sara.ristic.f@gmail.com, milos.mosic@ff.bg.ac.rs, marijapetkovic@ff.bg.ac.rs, katarinamiletic@ff.bg.ac.rs

[2]University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, 11000 Belgrade, Serbia, stefan.lekic@ihtm.bg.ac.rs

ORIGINAL SCIENTIFIC PAPER

ISSN 2637-2150
e-ISSN 2637-2614
UDC 616.831-085.4:582.573.16
DOI 10.7251/STEDZ2402001R
COBISS.RS-ID 141778945

Received: *18 July 2024.*
Accepted: *01 November 2024.*
Published: *29 November 2024.*
http://stedj-univerzitetpim.com

Corresponding Author:
Katarina M. Miletić, University of Belgrade, Faculty of Physics, Studentski trg 12-16, 11001 Belgrade, Serbia, katarinamiletic@ff.bg.ac.rs

Citation:
Ristić, S.V., Mošić, M.S., Petković Benazzouz, M.M., Lekić, S., & Katarina, M.M. (2024). Advancing plant metabolism analysis: a real-time optical approach, insights from Variesea carinata Wawra. *STED Journal*, *6*(2), 1-9.

ABSTRACT

Optical detection of plant stress in real-time is crucial as it enables timely interventions to mitigate potential damage. This study presents a detailed evaluation of a system that detects changes in plant metabolism in real-time by distributing optical signals across the leaf. The methodology facilitates continuous monitoring of changes in the optical properties of plant leaves through measurements of optical transmission coefficients using a 665 nm LED light signal, thereby recording the circadian rhythm over time. Given that the photosynthetic processes within the leaves are closely linked to the plant's overall health, this system can detect stress caused by various factors and identify metabolic changes by analysing the circadian rhythm patterns of the observed plants.

For inducing metabolic changes, the plant *Vriesea carinata Wawra*, a verified representative of dual metabolism, was subjected to high light intensity stress. To validate the method, the collected results were compared with data obtained through chemical methods to establish a correlation between the traditional, destructive method and the non-destructive, optical method.

The findings successfully identify circadian rhythms as parameters for recognizing changes in plant metabolism, demonstrating the significance of the proposed method in researching plant physiology through the optical identification of biological processes.

Keywords: Plant metabolism, Circadian rhythm, Non-destructive method, Real-time optical approach, Stress detection.

INTRODUCTION

Modern plant cultivation practices require continuous monitoring of various parameters affecting plant health. Early stress detection is crucial for predicting the success of growing particular plant species (Shah, Houborg, & McCabe, 2017). Identifying plant stress is essential for improving agricultural practices and preserving plant resources. According to the United Nations, climate change leads to significant soil degradation and air composition changes, indirectly impacting food quality (Rumbaitis, & Guilanpour, 2023). Effective monitoring of plant physiological status can significantly reduce agricultural losses, enhance yields, and minimize the use of chemical agents.

Traditional approaches to monitoring plant health often rely on time-consuming, subjective, and destructive methods. These methods involve physicochemical analyses of plant tissues, requiring detailed sample preparation (Kvet, Ondok, Necas, & Jarvis, 1971). It is well established that a plant's overall health correlates with the intensity of all photosynthetic processes in its leaves (Knapp, & Carter, 1998). To achieve continuous monitoring of vital plant processes, non-invasive methods are increasingly employed, providing insights into plant health and metabolic changes by measuring the spectral characteristics of leaves throughout the growth and life cycle. These methods are based on specific wavelengths of light used to measure optical properties of plant leaves, such as transmission and reflection (Gitelson, Gritz & Merzlyak, 2003; Combes et al., 2007; Liu, & van Iersel, 2021).

A novel method in this field has been developed at the Faculty of Physics, University of Belgrade, within the Department of Applied Physics and Metrology. This method enables continuous real-time monitoring of the physiological and health status of plants and their metabolic processes (Kasalica et al., 2021). This innovative system uses specific spectral responses of plant leaves to appropriate stimuli necessary to define optimal conditions for the development and growth of certain plant species. By tracking circadian rhythms, this method allows early detection and precise identification of stress states caused by factors such as changes in light intensity, nutrient deficiencies, herbicide use, or pathogen presence (Miletic et al., 2022; Miletic, Mošic, Ristic, & Petkovic-Benazzouz, 2023; Veljovic Jovanovic et al., 2023).

One of the critical aspects of monitoring plant metabolism is detecting the transition between C3 and CAM metabolism. C3 plants use the Calvin-Benson cycle for carbon dioxide fixation, while CAM plants assimilate carbon dioxide at night to reduce water loss during the day. The transition between these two types of metabolism is a significant indicator of plant adaptation to stress conditions, such as intense light exposure (Maxwell et al., 1995; Winter & Holtum, 2021). Understanding and detecting this transition is crucial for researching plant adaptive mechanisms and their responses to environmental changes. Through precise analysis of the spectral responses of leaves to various external factors, this method offers a more accurate insight into plant adaptive mechanisms, enabling the development of effective strategies to improve plant health and productivity.

The aim of this study is to investigate the correlation between the circadian rhythm function obtained using the non-invasive optical method and the concentrations of malic and citric acids after exposing plants to light stress.

MATERIAL AND METHODS OF WORK

Plant Material

A group of five *Vriesea carinata Wawra* plantlets, all genetically identical clones, was cultivated in a controlled environment within a growth chamber (Figure 1). The conditions were meticulously regulated, maintaining a constant temperature of 22/23°C (day/night), a 12/12-hour photoperiod, relative humidity around 70%, and a light intensity of approximately 140 µmol/s/m².

Figure 1. *Vriesea carinata Wawra* seedlings used in the experiment

Stress Induction by Light Intensity Variation

To induce stress through changes in light intensity (Group A), an initial set of five *Vriesea carinata Wawra* plantlets was exposed to light at an intensity of PPFD = (140±30) µmol/s/m². Over the subsequent three days, the light intensity was increased to PPFD = (850±60) µmol/s/m². This adjustment was implemented to simulate stress caused by a sudden change in light intensity, thereby triggering the transition of the plant from C3 to CAM metabolism.

Chemical Method for Determining Malic Acid Concentration

For the chemical determination of malic acid concentration, leaf samples were collected at night before stress and after stress. A single leaf from each plant was immediately immersed in liquid nitrogen, then ground into a fine powder. Two grams of each sample were mixed with 10 ml of 80% (v/v) aqueous ethanol solution. After homogenizing the mixture for one minute, the samples were subjected to ultrasonic treatment for 30 minutes. The mixture was then centrifuged at 3,000 rpm for 5 minutes. Post-centrifugation, 100 µl of the supernatant was diluted with 900 µl of the mobile phase (10% solution A and 90% solution B), and filtered through a 0.45 µm Nylon filter. Finally, 20 µl of the filtrate was analyzed using liquid chromatography (LC) (Fernández-Fernández, et al., 2010).

NOM for Displaying Circadian Rhythm

In this experiment, the Non-invasive Optical Method (NOM) described in detail in Kasalica et al. (2021) was utilized. This method enabled the monitoring of the circadian rhythm of light transmission through the leaves of *Vriesea carinata Wawra*.

The system consists of 20 identical segments, each corresponding to a measurement point where a plant leaf sample is placed. For the growth chamber housing the *Vriesea carinata Wawra* plants, 10 measurement points were designated, with two measurement points per plant, resulting in a total of 10 measurement points for the five plants. This setup ensured the acquisition of statistically reliable data on the circadian rhythm.

Each segment of the system includes a leaf holder made of transparent plexiglass, allowing for movement in six degrees of freedom to accommodate the natural movement of the leaf (Figure 2).

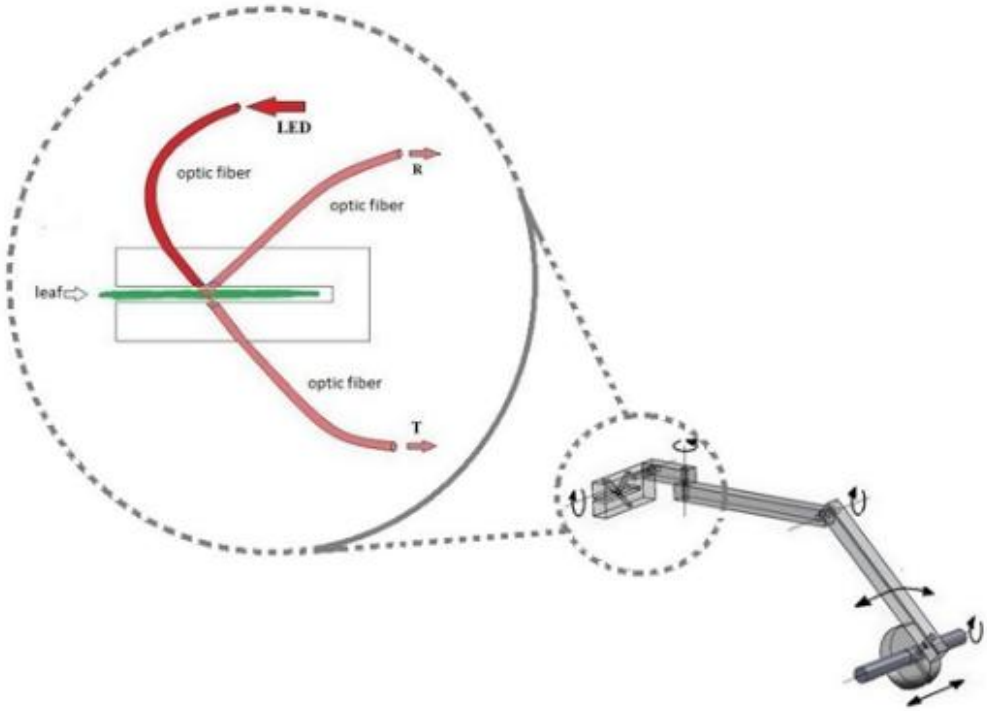

Figure 2. Single measurement channel (Kasalica et al., 2021)

The holder has three slots for optical fibers, positioned at a 45-degree angle relative to the plane of the leaf. These optical fibers are used to illuminate the leaf with an LED (corresponding to the wavelength of maximum chlorophyll-a absorption at 665 nm), collect reflected light, and collect transmitted light, respectively. One end of the fiber optic cable is placed near the leaf, while the other end transmits the signal to an appropriate photodiode. Above the photodiode surface, a filter is placed to allow only light of a specific wavelength (corresponding to the LED wavelength of 665 nm) to pass through. A focusing lens ensures that the light beam is concentrated on a small area to prevent light scattering.

The photodiodes send signals to a measurement device (I/O card), and using appropriate electronics, a graph of the transmission coefficient over time is plotted, depicting the circadian rhythm (Kasalica et al., 2021).

Statistical Analysis

To determine the relationship between optical measurements (transmittance) and biochemical parameters (total acidity), the Pearson correlation coefficient was used. This coefficient quantifies the linear dependence between two variables, where values range from -1 to 1, indicating the strength and direction of the relationship.

In this study, transmittance was measured as part of the optical analysis before and after light stress, while total acidity was assessed through chemical analysis of the plant leaves. The correlation coefficient was used to determine whether a significant linear relationship existed between these changes in the plant, considering the relationship between transmittance at night and during the day, before and after the transition from C3 to CAM metabolism.

RESULTS AND DISCUSSION

Results Obtained by Non-invasive Optical Method

One of the results illustrating the circadian rhythm obtained using the non-invasive optical method is shown in Figure 3. This figure demonstrates the temporal variation in light transmission through the leaves of *Vriesea carinata Wawra*, highlighting the effectiveness of the method in monitoring circadian rhythms in real-time.

All five samples in both groups exhibit the same trend in the circadian rhythm function, with only differences in the percentage of transmission, which depends on the position and thickness of the leaf relative to the fiber optic cable and leaf holder. Therefore, only one graph per group is presented.

Analysis of the results obtained using the non-invasive optical method reveals significant differences in the circadian rhythm function of plants due to changes in light conditions, covering a period of three days before and four days after the increase in light intensity. Under normal conditions, a consistent circadian rhythm was recorded, characterized by a decrease in transmission during the day (Figure 3), indicating increased absorption.

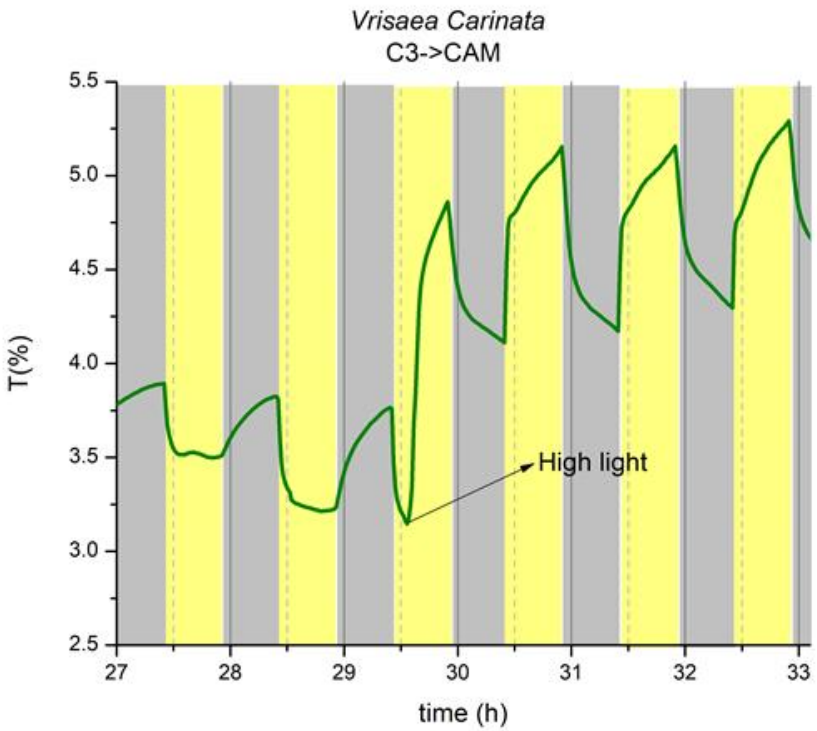

Figure 3. Circadian rhythm of plants (change in light intensity)

After the change in light intensity, a complete inversion in the circadian rhythm of light transmission was observed, with transmission being lowest during the night (gray area in Figure 3), suggesting a metabolic shift as the plants minimize water loss through stomata during daytime heat.

Interestingly, smaller peaks were recorded at times when the lights were turned on or off. These changes effectively demonstrate how plants respond to alterations in their light environment, providing clear evidence of the spectral response of plants to modified lighting conditions.

Results Obtained by Chemical Method

The results obtained by the chemical method are presented in Figures 4a and 4b. These figures illustrate the measured concentrations of malic acid in the leaf samples under different conditions.

The obtained results show diurnal fluctuations in the concentrations of these acids, which align with the known patterns for plants utilizing CAM and C3 metabolism. For CAM plants, the concentration of malic acid is significantly higher at night when CO_2 is fixed in the form of organic acids while the stomata are open, and lower during the day when these acids are broken down. This is clearly visible in our results, where CAM plants exhibit high nighttime values of malic and citric acids that decrease during the day (Haag-Kerwer, Franco, & Luttge, 1992). Conversely, C3 plants do not show significant fluctuations in malic acid concentration. Our data confirm this pattern, as the concentration of malic acid in C3 plants remains relatively stable throughout the day and night (Haag-Kerwer, Franco, & Luttge, 1992).

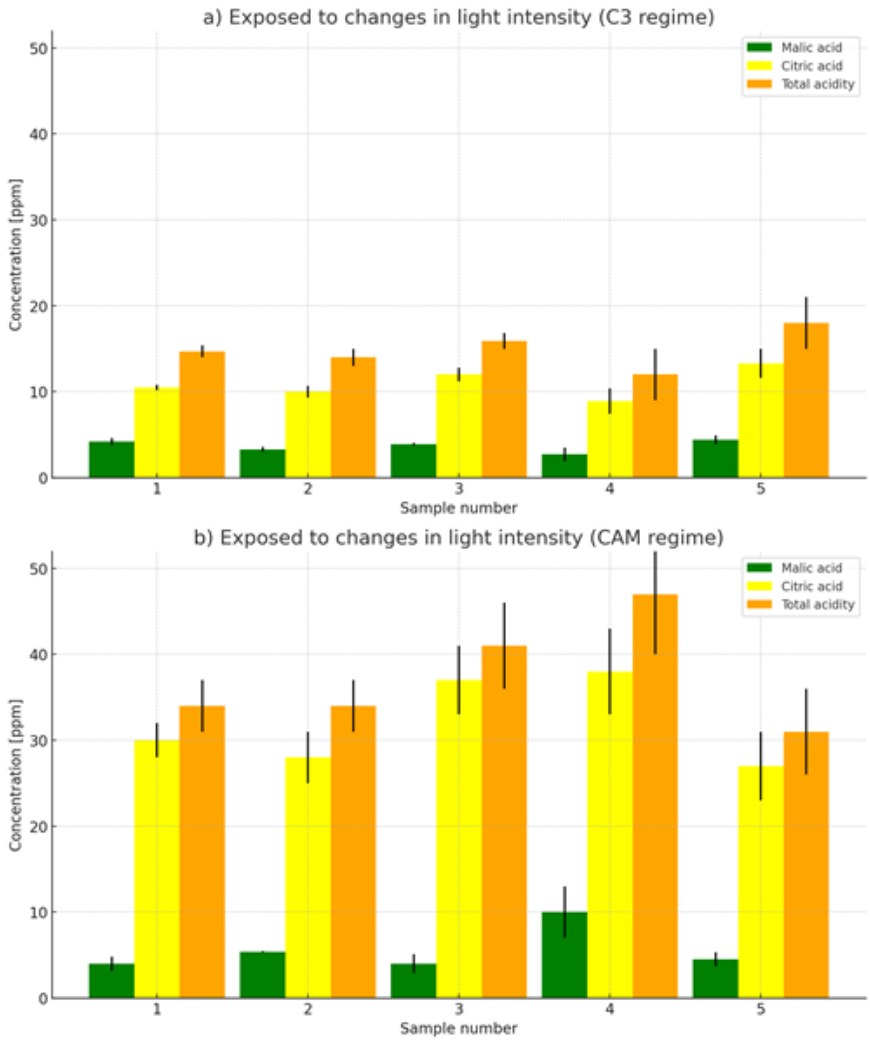

Figure 4. Concentration of malic and citric acid in *Vriesea carinata Wawra* samples
a) C3 regime b) CAM regime

Statistical Analysis of the Correlation Between Optical and Chemical Data

To provide further insight into the relationship between the changes observed through the non-invasive optical method and the biochemical analysis, we calculated the Pearson correlation coefficient between the ratio of transmittance during the night and day (max/min ratio) and the total acidity values before and after increasing light intensity.

The calculated Pearson correlation coefficient was $r = -0.956$, indicating a strong negative linear correlation between the changes in light transmittance and the total acidity in the leaves of *Vriesea carinata Wawra*. This suggests that as transmittance decreases during the day (indicating increased absorption and CAM activity), the total acidity significantly increases, which is consistent with the expected physiological response during CAM metabolism.

These results emphasize the strong negative correlation, highlighting the reliability of the non-invasive optical method in detecting metabolic changes and confirming its agreement with chemical analysis. The estimated uncertainties were considered for both transmittance ratios and acidity measurements, ensuring a comprehensive understanding of variability in the data.

Our hypothesis about plant metabolism based on the non-invasive optical method aligns with the results obtained from chemical analyses. The study by Herppich, von Willert, & Herppich (1995) demonstrates similar fluctuations in the concentration of organic acids in plants transitioning from C3 to CAM metabolism under stressful conditions, such as drought or high light intensity (Herppich, von Willert, & Herppich, 1995). This consistency supports the reliability of our findings and underscores the effectiveness of the non-invasive optical method in monitoring metabolic changes in plants.

Furthermore, it is important to note that studies involving CAM physiology, particularly those focusing on metabolic changes under varying light intensities, often use a relatively small sample size due to the complexity and controlled nature of the experiments (Haag-Kerwer, Franco, & Luttge, 1992). Similar approaches have been documented in the literature, where small sample sizes are used to achieve in-depth physiological insights with precision under carefully regulated conditions. This highlights that our study design, despite the limited number of samples, is in line with the best practices for research of this type and provides robust evidence of the observed metabolic shifts.

CONCLUSIONS

This study provides the first evidence that plant metabolism can be determined non-destructively through the application of the Non-invasive Optical Method (NOM) on *Vriesea carinata Wawra*. The method was validated using chemical analysis of leaf acidity, demonstrating a strong correlation ($r = -0.956$) between optical and chemical data, confirming the reliability of the NOM technique.

The application of NOM demonstrates the ability of plants to respond to variations in light conditions, contributing to a better understanding of their adaptive strategies. The analysis of circadian rhythms, in particular, offers insights into daily oscillations in metabolic activity and their connection to the plants' ability to adapt to changing environmental conditions. Similar findings regarding circadian rhythm fluctuations have been observed by Herppich, von Willert, & Herppich (1995), providing further validation of our observations.

Our study revealed that as transmittance decreased during the day, indicating increased light absorption and CAM activity, total acidity in the leaves significantly increased. This metabolic shift, which aligns with the expected physiological response in CAM plants, was captured in real-time using NOM. The consistency of these results with the chemical analysis highlights the precision of this method.

This work not only confirms the efficacy of NOM for real-time detection of changes in plant metabolism but also allows for more precise determination of optimal growth conditions regarding light intensity. By using chemical methods to validate NOM results, the data obtained are highly reliable and

provide a comprehensive view of plant adaptive mechanisms.

Furthermore, given the controlled nature and complexity of experiments involving CAM physiology, the limited number of samples in our study is in line with the best practices documented in the literature (Haag-Kerwer, Franco, & Luttge, 1992). This emphasizes that our findings are robust despite the limited sample size, and effectively demonstrate the potential of NOM for broader application.

NOM can be used for non-destructive analysis of the metabolism of various plants, including those for which chemical analysis has not yet been performed. This method enables the rapid formation of a database with clear classification of C3, CAM, and dual metabolism, significantly aiding researchers in this field to obtain relevant results more quickly than with traditional chemical methods.

In summary, this research represents a significant advancement in the use of optical methods for plant monitoring, particularly for studying CAM physiology under light stress conditions. The NOM technology opens new possibilities for more efficient monitoring and management of plant health in both natural and controlled environments. The results presented here are of great importance for future research in botany, ecology, and agriculture, paving the way for novel approaches to plant stress detection and resource optimization.

LITERATURE

Combes, D., Bousquet, L., Jacquemoud, S., Sinoquet, H., Varlet-Grancher, C., & Moya, I. (2007). A new spectrogoniometer to measure leaf spectral and directional optical properties. *Remote Sensing of Environment, 109*(1), 107–117. https://doi.org/10.1016/j.rse.2006.12.007

Fernández-Fernández, R., López-Martínez, J., Romero-González, R., Martínez-Vidal, J., Flores, M., & Frenich, A. (2010). Simple LC–MS Determination of Citric and Malic Acids in Fruits and Vegetables. *Chromatographia, 72*, 55-62. https://doi.org/10.1365/s10337-010-1611-0

Gitelson, A., Gritz, Y., & Merzlyak, M. (2003). Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. *Journal of Plant Physiology, 160*(3), 271-282. https://doi.org/10.1078/0176-1617-00887

Haag-Kerwer, A., Franco, A. C., & Luttge, U. (1992). The Effect of Temperature and Light on Gas Exchange and Acid Accumulation in the C3-CAM Plant Clusia minor L. *Journal of Experimental Botany, 43* (248), 345-352. https://doi.org/10.1093/jxb/43.3.345

Herppich, M., von Willert, D. J., & Herppich, W. B. (1995). Diurnal Rhythm in Citric Acid Content Preceded the Onset of Nighttime Malic Acid Accumulation during Metabolic Changes from C3 to CAM in Salt-stressed Plants of Mesembryanthemum crystallinum. *Journal of Plant Physiology, 147*, 38-42. https://doi.org/10.1016/S0176-1617(11)81409-4

Kasalica, B. V., Miletic, K. M., Sabovljevic, A. D., Vujicic, M. M., Jeremic, D. A., Belca, I. D., & Petkovic-Benazzouz, M. M. (2021). Nondestructive optical method for plant overall health evaluation. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science, 71*(9), 1017–1023. https://doi.org/10.1080/09064710.2021.1928740

Knapp, A. K., & Carter, G. A. (1998). Variability in leaf optical properties among 26 species from a broad range of habitats. *American Journal of Botany, 85*(7), 940–946. https://doi.org/10.2307/2446360

Kvet, J., Ondok, J. P., Necas, J., & Jarvis, P. G. (1971). Methods of Growth Analysis. In Z. Sesták, J. Catsky, & P. G. Jarvis (Eds.), *Plant Photosynthetic Production: Manual of Methods* (pp. 343-391). Springer.

Liu, J., & van Iersel, M. W. (2021). Photosynthetic Physiology of Blue,

Green, and Red Light: Light Intensity Effects and Underlying Mechanisms. *Frontiers in Plant Science, 12*. https://doi.org/10.3389/fpls.2021.619987

Maxwell, C., Griffiths, H., Borland, A. M., Young, A. J., Broadmeadow, M. S. J., & Fordham, M. C. (1995). Short-term Photosynthetic Responses of the C3-CAM Epiphyte Guzmania monostachia var. monostachia to Tropical Seasonal Transitions Under Field Conditions. *Functional Plant Biology, 22*, 771-781.

Miletic, K. M., Djunisijevic-Bojovic, D. M., Kasalica, B. V., Milutinovic, M., Petkovic-Benazzouz, M. M., Milanovic, S. D., & Jeremic, D. A. (2022). Innovative optical method for sensing the nutritional stress in hydroponically cultivated plants. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science, 72*(1), 720–732. https://doi.org/10.1080/09064710.2022.2071761

Miletic, K. M., Mošic, M. S., Ristic, S. V., & Petkovic-Benazzouz, M. M. (2023). Early detection of phytophthora plurivora pathogen infection in sweet chestnut leaves using nondestructive optical method. In *XII International Conference of Social and Technological Development – STED (2023), Trebinje* (pp. 15-18). ISSN 2637-3298.

Rumbaitis, C., & Guilanpour, K. (2023). *Options for a Politically-Salient Headline for the Global Goal on Adaptation (Technical Paper).* UN Foundation, Center for Climate and Energy Solutions. Retrieved from https://www.c2es.org/

Shah, S. H., Houborg, R., & McCabe, M. F. (2017). Response of chlorophyll, carotenoid and SPAD-502 measurement to salinity and nutrient stress in Wheat (Triticum aestivum L.). *Agronomy, 7*(3), 61. https://doi.org/10.3390/agronomy7030061

Veljovic Jovanović, S., Kasalica, B., Miletic, K., Vidovic, M., Susic, N., Jeremic, D., & Belca, I. (2023). Red-Light Transmittance Changes in Variegated Pelargonium zonale—Diurnal Variation in Chloroplast Movement and Photosystem II Efficiency. *International Journal of Molecular Sciences, 24* (18), 14265. https://doi.org/10.3390/ijms241814265

Winter, K., & Holtum, J. A. M. (2021). CAM photosynthesis: the acid test. *New Phytologist, 231* (4), 1485-1490. https://doi.org/10.1111/nph.17556