METROLOŠKA EVALUACIJA SISTEMA ZA OPTIČKO PRAĆENJE METABOLIZMA BILJAKA U REALNOM VREMENU

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Ključne reči: Transmisija u listu, Refleksija u listu, 665 nm LED, Optičke metode, Cirkadijumski ritam

KRATAK SADRŽAJ

U ovom radu izvršena je detaljna metrološka evaluacija sistema za praćenje metabolizma biljaka u realnom vremenu putem optičkog monitoringa. Predstavljena metodologija omogućava neprekidno praćenje promena optičkih svojstava listova biljaka preko merenja koeficijenata optičke transmisije i refleksije pomoću svetlosnog signala LED-a od 665 nm. Povezanost između različitih konstruktivnih elemenata aparature, kao što su: poli(metil metakrilat), optička vlakana, fotodiode, i LED paneli, obezbeđuje visok nivo preciznosti i pouzdanosti dobijenih rezultata. Proces kalibracije, detaljno usklađen sa metrološkim standardima, osigurao je tačnost i konzistentnost rezultata. Kalibracija je podrazumevala pojedinačan proračun mernih mesta po vrednostima optičke transmisije i refleksije u procentima na osnovu etaloniranih neutralnih filtera, različite optičke gustine, odnosno etalona za difuznu refleksiju, respektivno. Dobijeni rezultati ukazuju na uspešnu identifikaciju cirkadijumskih ritmova i odgovora biljaka na varijabilne tretmane, demonstrirajući značaj predložene metode u istraživanju fiziologije biljaka, čime se otvaraju nove mogućnosti za primenu u poljoprivredi. Ovaj sistem pruža čvrstu osnovu za dalja istraživanja uslova rasta, doprinoseći time dubljem razumevanju bioloških i ekoloških procesa koji utiču na vitalnost biljaka.

METROLOGICAL EVALUATION OF A SYSTEM FOR REAL-TIME OPTICAL MONITORING OF PLANT METABOLISM

Keywords: Leaf Transmittance, Leaf Reflection, 665 nm LED, Optical Methods, Circadian Rhythm of Plants symposium, word processing, proceedings, styles

ABSTRACT

In this work, a detailed metrological evaluation of the system for real-time monitoring of plant metabolism through optical monitoring was conducted. The presented

methodology allows for the continuous observation of changes in the optical properties of plant leaves through the measurement of optical transmission and reflection coefficients using a 665 nm LED light signal. The connection between various constructive elements of the apparatus, such as poly(methyl methacrylate), optical fibers, photodiodes, and LED panels, ensures a high level of precision and reliability of the obtained results. The calibration process, meticulously aligned with metrological standards, ensured the accuracy and consistency of the results. Calibration involved an individual calculation of measuring points by values of optical transmission and reflection in percentages based on calibrated neutral filters of different optical densities, or standards for diffuse reflection, respectively. The obtained results indicate successful identification of circadian rhythms and plant responses to variable treatments, demonstrating the significance of the proposed method in the research of plant physiology, thereby opening new possibilities for application in precision agriculture. This system provides a solid foundation for further research into growth conditions, contributing to a deeper understanding of the biological and ecological processes that affect plant vitality.

INTRODUCTION

Understanding plant metabolism in real-time is crucial for advancing agricultural practices and ensuring optimal plant health. In recent years, the field of precision agriculture has seen a surge in the development of non-invasive techniques to monitor plant growth and physiological states. Among these, optical methods have emerged as particularly effective, offering detailed insights into plant health without causing any harm.

Optical monitoring techniques utilize specific wavelengths of light to measure the properties of plant leaves, such as transmission and reflection. These measurements can reveal a lot about the plant's metabolic state and circadian rhythms, which are essential for understanding how plants respond to their environment [1, 2].

One of the significant advancements in this field is the use of light-emitting diodes (LEDs) at specific wavelengths, like 665 nm, which provide precise data on the optical properties of plant leaves. The integration of materials such as poly(methyl methacrylate) (PMMA), along with optical fibers and photodiodes, has further enhanced the accuracy and reliability of these systems. PMMA is particularly valued for its optical clarity and stability, making it a preferred choice for constructing optical components [3].

Calibration is a critical step in ensuring that these optical systems provide accurate and consistent data. By adhering to metrological standards and using calibrated neutral filters and diffuse reflection standards, we can achieve precise measurements of optical transmission and reflection in plant leaves [4].

In this study, we conducted a comprehensive metrological evaluation of a system designed for real-time optical monitoring of plant metabolism. By using a 665 nm LED light signal, we were able to continuously observe changes in the optical properties of plant leaves. Our calibration process, rigorously aligned with metrological standards, ensured the precision and consistency of our results. This system successfully identified the circadian rhythms of plants and their responses to various treatments, demonstrating its potential applications in precision agriculture [5-11].

MATERIALS AND METHODS

System Operation

The plant monitoring system consists of 20 identical segments arranged in two groups of 10, each serving as a measurement site for a plant leaf sample. The leaf holder, made from transparent plexiglass, offers 6 degrees of freedom to accommodate natural leaf movements. It includes three slots for optical fibers positioned at a 45-degree angle to the leaf plane. These fibers illuminate the leaf with a 665 nm LED (matching chlorophyll-a absorption) and collect reflected and transmitted light.

One end of the optical fiber is near the leaf, while the other transmits the signal to a photodiode, converting the light signal into an electrical signal. A filter above the photodiode allows only specific wavelengths to pass through, and a collecting lens minimizes light dispersion, directing it to the recording device.

Photodiodes, signal LEDs, and associated electronics are housed in a thermostated and insulated box to protect them from external influences. Signals from the photodiodes are sent to an I/O card, which, along with appropriate electronics and software, provides the LED power supply and continuous measurement capabilities. Based on these measurements, graphs illustrating the plant’s circadian rhythm and overall condition are plotted.

During experiments, a live plant leaf is placed in a holder segment, allowing unimpeded growth in 6 degrees of freedom. The setup includes 20 independent channels, each corresponding to a segment. Each holder uses one optical fiber to deliver a signal from the LED (665 nm), with the remaining fibers collecting reflected and transmitted light. The collected light is transmitted to photodiodes equipped with interference filters (665 nm), as shown in the block diagram in Figure 1.

LED sequences are computer-controlled via an I/O card, directing signals through an analog multiplexer. Software sets the recording cycle interval, typically 15 minutes, resulting in approximately 100 cycles per day. Data is stored in separate tables for each channel, and graphs of reflection and transmission coefficients over time reveal the plant's circadian rhythms [5, 6].

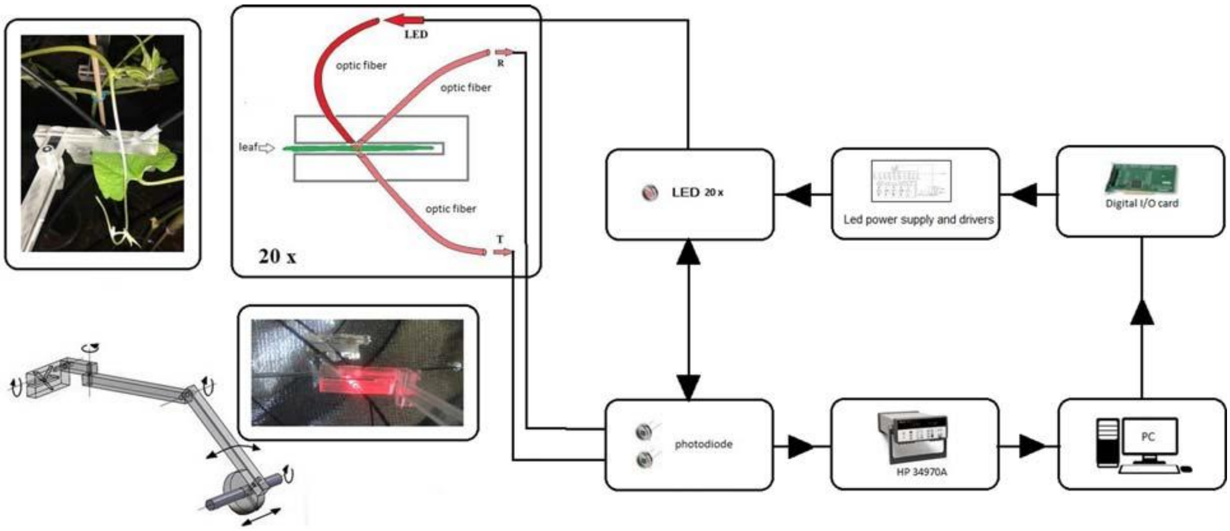

Figure 1. Block diagram [5]

The system allows for the adjustment of ambient conditions based on the specific plant group under study, ensuring optimal experimental conditions.

This setup allows precise, continuous monitoring of plant health and growth, providing valuable insights into circadian rhythms and overall plant condition. During the experiment, ambient conditions such as temperature and relative humidity are maintained. Light panel intensity is controlled with a luxmeter and spectrophotometer.

Calibration of Measurement Sites by Optical Transmission Value

Calibration is performed using calibrated neutral filters of different optical densities. Since the optical fiber collecting transmitted light is positioned at a 45-degree angle, calibration of neutral filters is conducted under the same geometry using a control spectrophotometer. The electrical signal value from the photodiode considered as 100% optical transmission is taken when the sample holder is empty, with no obstacles between the LED and the transmitted light fiber [6]. Transmission values recorded on the control spectrophotometer are used for calibration. A set of filters with different transmittance values (T_1 = 2.68%, T_2 = 8.54%, T_3 = 26.79%, T_4 = 67.42%, T_5 = 100%) was utilized to obtain varying signal dependencies measured in volts for each channel individually, for both the sample group and the control group, each containing 10 channels.

Calibration of Measurement Sites by Optical Reflection Value

The reflection of incident light on the plant leaf is diffuse, and calibration is done using diffuse reflection standards. The value of optical reflection lies between two extremes: specular reflection on a perfectly smooth surface and diffuse reflection on a perfectly matte surface. The role of these reflections is determined by the material's absorption coefficient. Standards made from MgO, $MgCO_3$, and $BaSO_4$ are closest to the “absolute white body.” The electrical signal value considered as 100% diffuse reflection is taken from samples made from matte white surfaces of MgO and $BaSO_4$.

$BaSO_4$ samples with approximately 50% reflectivity were also used for calibration [6].

Calibration of System Sensitivity

Calibration of system sensitivity involves defining clear measurement result boundaries between noise current and photodiode saturation.

The system is designed with four groups of 10 optical fibers (one group for reflection and 10 for transmission). Despite the photodiode being housed in a dark box with fiber bundle ends, its noise current is minimized. However, inactive fibers in the bundle can contribute to system noise due to daylight, resolved by placing a collecting lens and an interference filter on each photodiode surface, allowing only the LED signal wavelength.

Data collection occurs in two steps: recording the signal before LED activation (noise value) and during LED illumination. The difference represents the actual signal value.

Extended considerations and measurements under various operating conditions have led to the optimal positioning of the optical fibers relative to the leaf surface at a distance of 2 mm, as well as the adjustment of constant ambient conditions in the room, which results in cleaner signals and greater sensitivity and stability of the entire system [6].

Adjustment of Measurement System for LED Intensity and Illumination Period

To verify the LED radiation's impact on phytochrome stimulation, tests were conducted during a 3-second illumination interval. The light intensity should be strong enough for reliable transmission and reflection measurements but weak enough to avoid physiological changes in the leaf. Intensity adjustments are made by changing the power supply current. The optimal illumination interval was found to be 3 seconds with a light intensity of 1200 lx (22.3 μmol/m²), sufficient without affecting photochemical processes [6]. Spectrophotometer tests verified the absence of biochemical changes in the leaf during LED illumination, ensuring reliable measurement results.

RESULTS

Calibration Results of Optical Transmission and Reflection Values

Graphs illustrating the functional dependence of voltage on optical transmission coefficients for one group of 10 measurement sites are shown in Figure 2.

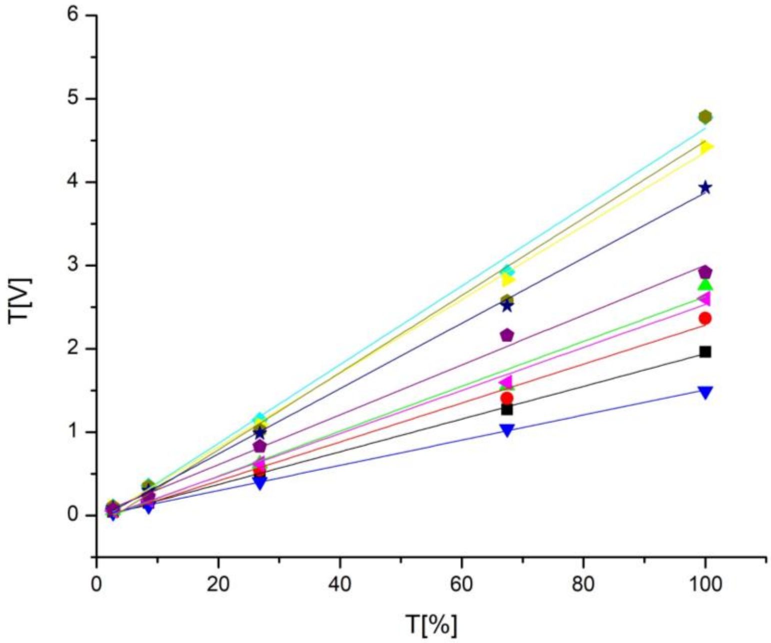

Figure 2. Calibration of optical transmission values [6]

The obtained fits are linear functions. Similar dependencies are observed for the second group and for both groups in terms of reflection. Each channel has its own unique linear equation with specific slope and intercept coefficients. Calibration is always performed before setting up new plants.

Circadian Rhythm Analysis

One of the graphs depicting the circadian rhythm of a plant growing under normal conditions is presented in Figure 3. This function represents the dependence of optical transmission over time, exhibiting a 24-hour period across several days of continuous measurement.

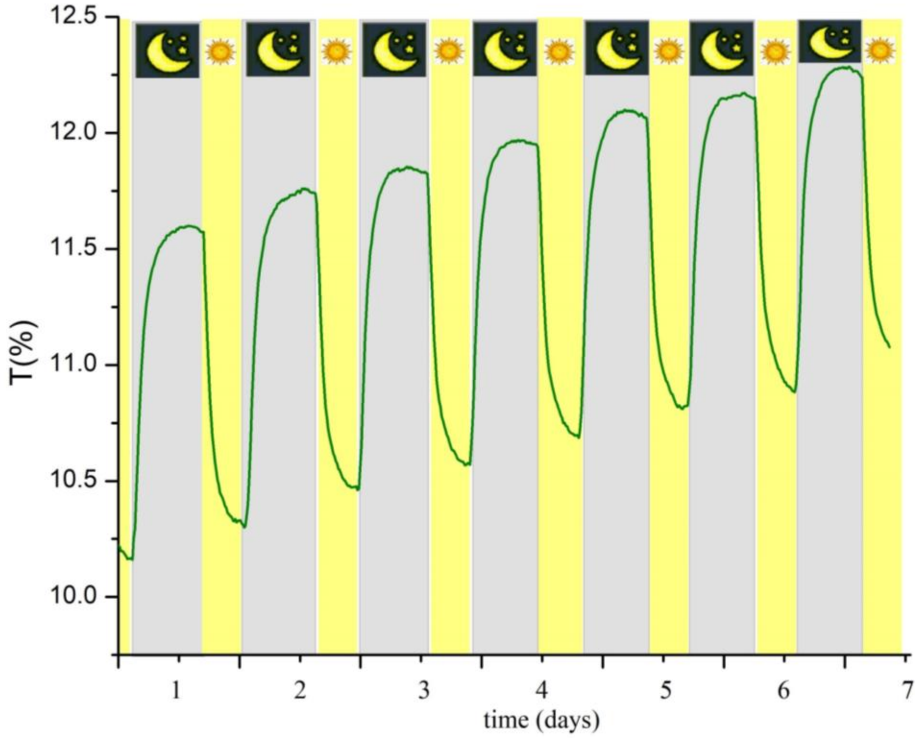

Figure 3. Circadian rhythm of a plant growing under normal conditions

The data clearly demonstrates a periodic function with a well-defined amplitude. This function can be mathematically modeled by the equation:

$$T(t) = A sin(\omega t + \varphi) + Bt + C \quad (1)$$

where T(t) represents the optical transmission percentage at time t, A is the amplitude of the sinusoidal function (reflecting the variation in transmission), ω is the angular frequency, φ is the phase shift (indicating the starting point of the cycle), B is the linear growth rate (showing the gradual increase in transmission due to plant growth), and C is the vertical shift (baseline transmission level).

Figure 4 illustrates the circadian rhythm of a plant subjected to drought conditions. Under these conditions, the circadian rhythm deviates from a regular pattern, exhibiting damped oscillations instead. This behavior indicates the plant's stress response over time, reflected in the optical transmission measurements.

This function can be mathematically modeled by the equation:

$$T(t) = A \cdot e^{-\beta t} \cdot cos(\omega t + \varphi) + C \quad (2)$$

where T(t) represents the optical transmission percentage at time t, A is the initial amplitude of the oscillations (reflecting initial transmission levels), β is the damping coefficient (indicating the rate of amplitude decay), ω is the angular frequency of the oscillations, φ is the phase shift (indicating the starting point of the cycle), and C is the baseline transmission level. This model captures the declining oscillatory behavior observed in the plant under drought stress, providing insights into its physiological responses.

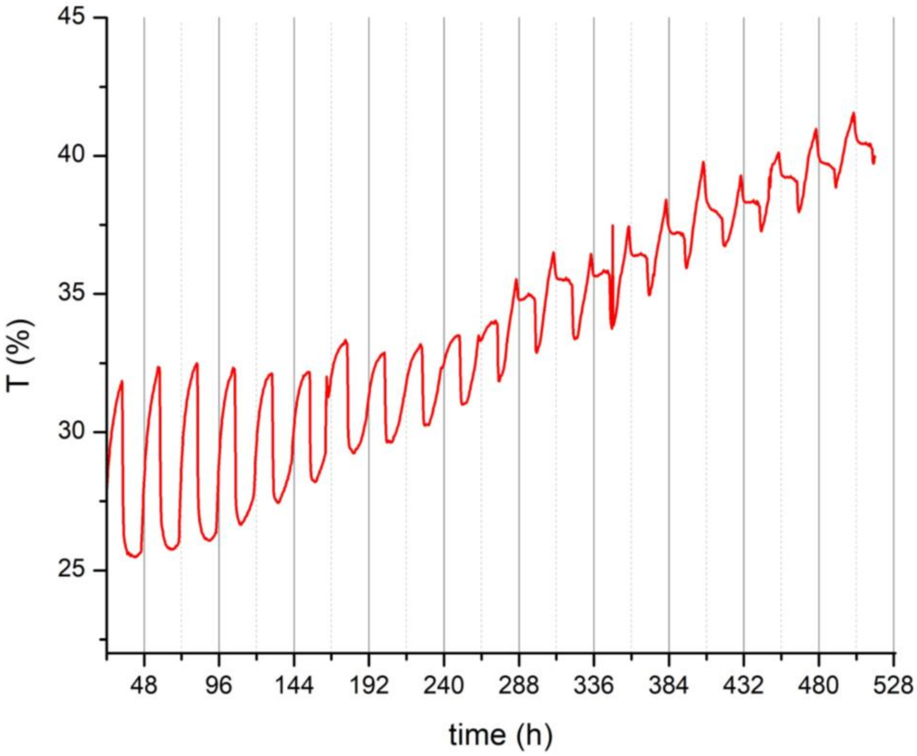

Figure 4. Circadian rhythm of a plant subjected to drought conditions

CONCLUSIONS

In this study, a detailed metrological evaluation of a system for real-time optical monitoring of plant metabolism was conducted. The system, incorporating 665 nm LEDs, optical fibers, and photodiodes, demonstrated high precision and reliability. Calibration procedures, rigorously aligned with metrological standards, ensured accurate measurements of optical transmission and reflection.

The successful identification of circadian rhythms and plant responses to various treatments highlights the system's potential applications in precision agriculture. This system provides a solid basis for further research into plant growth conditions, improving our understanding of the biological and ecological processes that impact plant health.

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