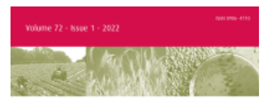

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Innovative optical method for sensing the nutritional stress in hydroponically cultivated plants

Katarina M. Miletic [a], Danijela M. Djunisijevic-Bojovic [b], Becko V. Kasalica [a], Marijana Milutinovic [b], Marija M. Petkovic-Benazzouz [a], Slobodan D. Milanovic [b,c], Ivan D. Belca [a], Mirjana Z. Sarvan[a] and Dejan A. Jeremic [d]

[a]Faculty of Physics, University of Belgrade, Belgrade, Serbia; [b]Faculty of Forestry, University of Belgrade, Belgrade, Serbia; [c]Faculty of Forestry and Wood Technology, Mendel University in Brno, Brno, Czech Republic; [d]Innovation Center of the Faculty of Chemistry, University of Belgrade, Belgrade, Serbia

ABSTRACT

Well-balanced nutrition is important for the successful cultivation of healthy plants. In this paper, we demonstrate a nondestructive optical method that can sense a deficiency of certain nutrients. The setup was tested on hydroponically grown *Ocimum basilicum*. The plants were subjected to nutrient deficiency by the exclusion of one of the essential elements (Fe, Mg, P, N) from the hydroponic solution. A control group of plants, fed by the balanced hydroponic solution, was also grown under the same conditions. The proposed method tracks and records the optical transmittance of the plants' leaves. All groups exhibit clearly defined day-night Circadian rhythms. When compared to the control group, the treated plants exhibited modified circadian rhythms of the optical transmission, suggesting an early indicator of the plants' stress. The condition of the plants under test was also assessed by the more common (destructive) methods such as: measurements of the determination of the photosynthetic pigment content, dray weight determination and the efficiency of PSII. Several biological parameters were observed, calculated and compared to the graphs of optical transmission dependence in real time. Presented results have demonstrated a significant potential of the proposed optical method for the early detection of plants' stress in hydroponic cultivation.

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Leaf transmittance; 665 nm red LED; Circadian rhythm; *Ocimum basilicum*; plant monitoring; nutrient deficiency

Introduction

The growing world population and the evident climate change are the major concerns regarding the survival of the human race. A recent study by Islam and Talukder (2017) has offered a robust prediction of the future global food demand. The study also predicts that the human population will reach 9.8 billion by the end of the year 2050. In addition, the greenhouse gasses emissions, and the associated shifts in temperature, as well as the diminishing fresh water supply, are all expected to impact the Earth's vegetation. Therefore, the future agricultural practices must adapt. In that context, it is important to use improved methods for the efficient and responsible use of the limited nutritional resources, such as phosphorus or nitrogen (Vance et al. 2003; Dawson and Hilton 2011; Alewell et al. 2020).

Contemporary approach to the plant cultivation involves constant monitoring of various parameters that affect the health of the crops. The evaluation of the plant stress provides a valuable predictor for the success of the cultivation effort. The stress is defined as the plant's response to the different external factors (stressors) and can be biotic (infections by plant pathogens) and abiotic (exposure to the different physical and chemical influences).

It is well-known fact that the general state of a plant is correlated with the intensity of all photosynthetic processes in its leaves (Fourty et al. 1996). Chlorophyll molecules provide the conversion of absorbed light into biochemical energy through the Calvin-Benson cycle (Porcar-Castell et al. 2014). In monitoring and managing of the plant stress, the reliable measurements related to leaf photosynthetic pigments are important elements (Shah et al. 2017). The quantity of chlorophyll and other photosynthetic pigments in a leaf is a good indicator of the plant's physiological status and can indicate if a plant is exposed to a stressor.

Photosynthetic pigments capture the light energy and provide the initial electrons for the electron transport chain. This is essential for the photochemical processes in photosynthesis. Consequently, the

CONTACT Katarina M. Miletić katarinamiletic@ff.bg.ac.rs Studentski Trg 12, 11000 Belgrade

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chlorophyll content is an important indicator of the plant photosynthetic capacity. Environmental stresses usually negatively influence the chlorophyll synthesis and may cause its degradation. This, in turn, causes a decrease in vegetation productivity. Carotenoids are the second photosynthetic pigment group that is important for the photosystems I and II and the antenna systems. As a part of LHC (light harvesting centres) they participate in: (i) harvesting the light energy, (ii) protection of the reaction centres of photosystems from the photooxidation (thought dissipation of the excess light energy) and (iii) the defence against the oxidative stress (Havaux 2014). Abiotic stressors such as the osmotic stress and the nutrient deficiency affect the photochemical and non-photochemical processes in photosynthesis, the pigment concentration ratio and the overall growth and development in higher plants (Kastori 1995; Pavlovic et al. 2014).

Determination of relative values of chlorophyll content using chlorophyll meters such as the SPAD-502 is a non-destructive approach based on measurements of the leaf optical transmittance in the red (650 nm) and infrared (940 nm) region. However, this technique necessitates a careful interpretation of the obtained results as the optical readouts are affected by the leaf structure and the local micromorphology at the measurement spot (Shah et al. 2017).

Chlorophyll fluorescence is yet another common technique used for the stress estimation in plants. It provides a detailed information about the state of photosystem II and its responses to the environmental changes. Measuring of minimum (*Fo*) and maximum (*Fm*) fluorescence, following the optimal period of dark adaptation, and the determination of photosynthetic efficiency (*Fo-Fm*)/*Fm*, is being used as the most common method to assess the stress in leaves (Murchie and Lawson 2013).

The idea to use reflectance spectroscopy for the non-destructive leaf chlorophyll examination is not a new one. It was first proposed in 1977 by Admas and Arkin. This approach was followed by numerous studies (Curran et al. 1995; Markwell et al. 1995; Datt 1999).

In our previous paper (Kasalica et al. 2021) we described a novel optical method capable of real-time tracking of plants' optical properties. The present paper is a practical application of the described method and an attempt to detect the nutritional stresses in hydroponically cultivated *Ocimum basilicum* (Basil).

Basil is a medicinal plant, rich in antioxidants and phenolic compounds, with strong antioxidant, anticancer, and antibacterial properties (Güez et al. 2017; Yanfei Zhan et al. 2020). It is well adapted to hydroponics and the controlled environment agriculture (CEA) systems, having a high profitability margin (Sipos et al. 2021). The optimal nutrient supply during plant's growth and development in a hydroponic system is of utmost importance for the plant's quality features (nutritional value, the concentration of bioactive compounds, biomass, etc.). Macronutrients and micronutrients are essential for maintaining the plant's optimal growth and health.

The iron is, for example, an important part in respiratory and electron transport system of a plant and participates in chlorophyll biosynthesis. While low iron concentration can seriously affect the plant growth (Vara et al. 2000; Dubyak 2004), in higher concentrations it can also be harmful, producing an imbalance between oxidants and antioxidants via Fenton reaction (Nikolic et al. 2019) and cellular damage. When a plant is iron-deficient one of the visible consequences is chlorosis. However, well before the onset of chlorosis, the plant has already experienced a significant nutritional stress. Nitrogen plays an important role in plant growth with regard to the synthesis of major molecules including proteins, nucleic acids, hormones, and chlorophyll (Hopkins and Hüner 2004) while magnesium affect photosynthetic performance, and cellular stress defence mechanisms (Hauer-Jákli and Tränkner 2019). The consequence of deficiency is reduction in plant growth based on dry matter and uniform (N) or interveinal (Mg) chlorosis on matured leaves under severe starvation while phosphorous is critical for plant growth and biomass portioning as a structural element of nucleic acids and phospholipids, which is crucial for energy metabolism, signalling and enzyim activation (de Bang et al. 2021).

Measuring the optical reflection and transmission as a method for plant health monitoring, provides the means for the early detection of the aforementioned stresses. However, it should be noted that the practical implementation of this method is followed by a few experimental challenges. The most important one is that, during its growth, the plant's leaves are changing their spatial position. In our previous paper (Kasalica et al. 2021) we have offered a solution to overcome this difficulty by designing an advanced measurement system. The system can track the optical transmission of plant leaves during their complete life circle, while simultaneously avoiding mechanical pressures on the plant; thus providing the growing conditions mimicking the nature. In this paper, we report the successful deployment of our method for monitoring the nutritional stress development in *Ocimum basilicum*.

A goal of the present work is to provide a device capable of contributing information on the presence any nutrition stress for the plant. In addition, this device should be practical to use, both in the laboratory and open fields.

Materials and methods

Experimental design

Recently, we have described a nondestructive measurement system which can track the optical transmission of the leaves in real-time dependence. In short: the experimental apparatus provides 20 independent 'channels', where each channel corresponds to a single leaf. For each channel, the source of light is a red Signal LED with the spectral emission maximum at 656 nm. Each Signal LED was coupled to an optical fibre. The other end of this fibre was attached to the appropriate leaf holder made of transparent plastic (methyl methacrylate).

Two additional optical fibres, one collecting transmitted and one collecting reflected light were also incorporated into the arrangement. The light collection fibres were also attached to a leaf holder.

The optical fibres transmit the collected light to the photodiodes which are kept in a thermostated shielded box. The signals from the photodiodes were routed through to the precision digital multimeter (DMM) (HP 34970A). The DMM was connected to the PC via RS-232 interface. The leaf holder was designed to enable 5 degrees of freedom for the mechanical movement, thus providing the means to track the leaf's position and orientation (Kasalica et al. 2021). The channels were distributed into six independent groups, where each group contains three single channels, but the two remaining channels were used to control the entire system dynamics with neutral filters. Each group was treated under different growing conditions (i.e. with different nutrient solutions):

Group I: Plants had continual growth in a complete nutrient solution control [C]

Group II: Plants were subjected to Fe deficiency by excluding Fe [–Fe]

Group III: Plants were subjected to Mg deficiency by excluding Mg [–Mg]

Group IV: Plants were subjected to P deficiency by excluding P [–P]

Group V: Plants were subjected to N deficiency by excluding N [–N]

Group VI: Plants were subjected to a deficiency caused by the nutrient exhaustion, *i.e.* the initial nutrient solution was never replenished during the plants' growth [–E].

The overall arrangements of the experiment were: the growing box (pot for one group of plants which contains three plants), the illumination panel and the garden box.

Growing boxes were hand-made of autothermic universal plate and covered by the aluminium reflex steam vapour dam. This cover also served as the light and thermal insulation. To reduce the risk of moving leaves, plastic hosepipes were used for change nutrient solutions every 3 days. An illustration of a single growing box is given in Figure 1.

The panel and the growing boxes were in the garden box. Two groups of plants were illuminated with a single panel with constant position during the experiment. In addition, the exterior of the garden box was made of advanced material that does not transmit light either outside or inside, while the interior is coated with mylar foil that reflects up to 98% of light. The plants were only light absorbers in the garden box.

PPFD distribution

We used three LED dimmable panels with continuous illumination (model: Samsung LED LM301H Quantum Tech V3 Panel Light 240W). The panels were mounted in a temperature-controlled box with specially designed fan system to manage the panel's heating. The illumination spectrum of the panels is given in Figure 2.

The plant lighting analyser (model: OHSP350P) was used for measuring PPFD values. We recorded the absolute measurements of the PPFD values for each channel which was attached to the plants' leaves at the beginning of the experimental set up. The PPFD mean value with standard deviation was PPFD = (221 ± 12) μmol/s/m^2. The PPFD map of the used LED panels with appropriate

Figure 1. Single growing box.

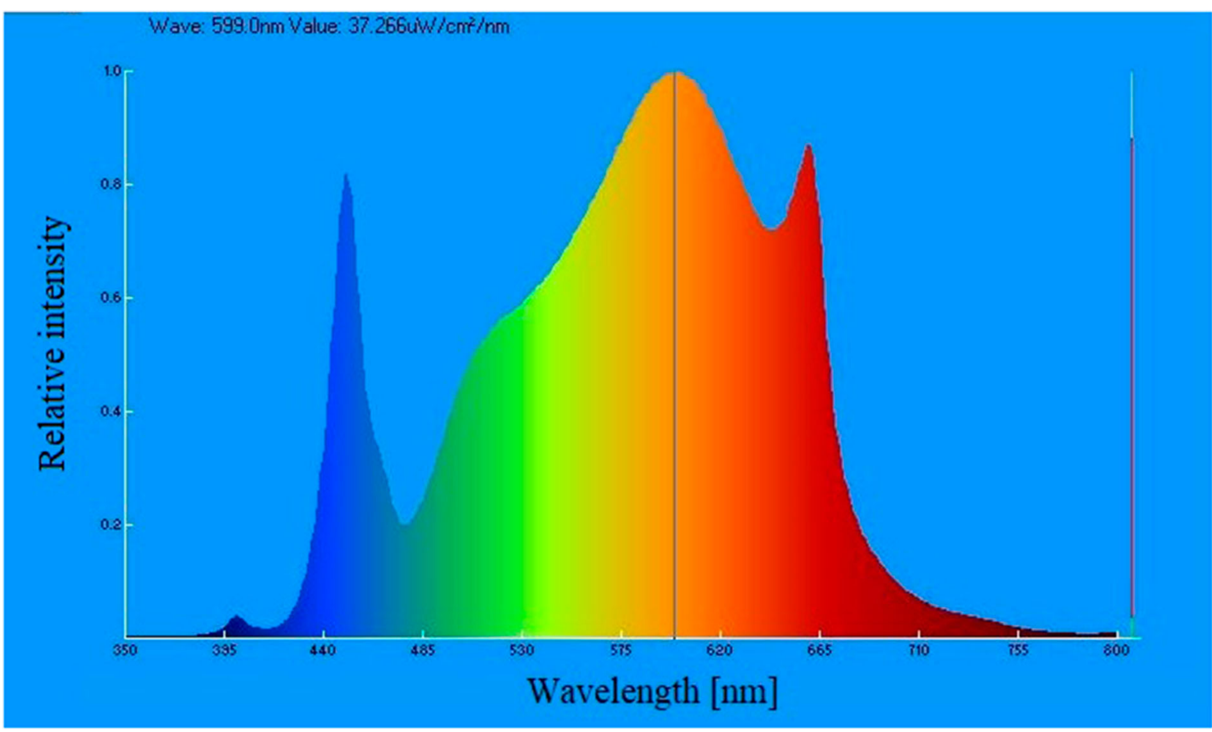


Figure 2. Light emission spectrum of the Samsung LED LM301H grow panel.

values in is given in Figure 3. For day to day checks it is much accessible to measure light intensity by calibrated data logger (Illuminometer photometer), which was in the clear-cut correlation with PPFD plant lighting analyser. The illuminometer was located in the centre under the led panel and collected data every 5 min during the experiment. The illuminometer mean value with standard deviation was: Illuminance = (19500 ± 200) lx.

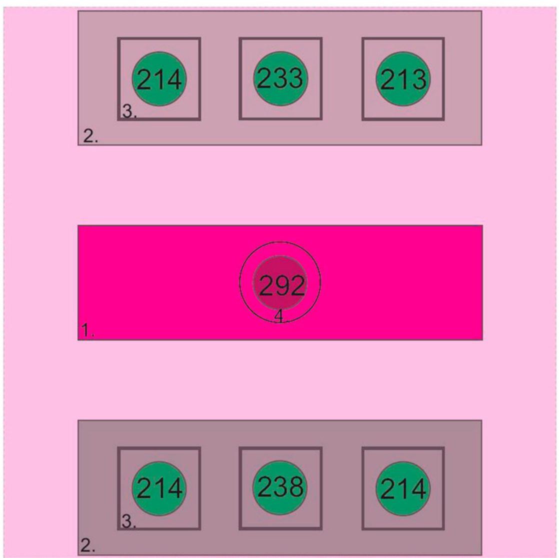


Figure 3. The PPFD distribution map: 1 – LED panel, 2 – Growing boxes, 3 – Plants, 4 – Illuminometer.

Plant material

The experiment was set up in an indoor environment. The growing chamber had the temperature regulation (25/20°C) and photoperiod (16/8 h), photosynthetic photon flux density of 200 μmolm^{-2}s^{-1}at plant heights, and humidity of~ 70%.

Ocimum basilicum plants were developed from the commercially obtained seeds. The seeds were germinated on moist filter papers at 25°C. At the stage of the first leaf development, the plants were transferred to half-strength modified Hoagland nutrient solution (ten plants per 3-L pot) in 6 pots. Final concentration of salts in the nutrient solution were (expressed in mmolL$^{-1)}$: 0.35 K_2SO_4, 0.05 KCl, 1.0 Ca $(NO_3)_2$, 0.25 $MgSO_4$, 0.05KH_2PO_4; and in μmol L^{-1}: 5 H_3BO_3, 0.25 $MnSO_4$, 0.25 $ZnSO_4$, 0.1 $CuSO_4$, 0.005 $(NH_4)_6Mo_7O_{24}$, and 20 Fe^{III}EDTA.

The plants were grown in the continuously aerated nutrient solutions. After four weeks in the complete solutions, the plants were transferred in 1L volume vials (one plant per vial) and connected to the 18 optical channels of our apparatus (3 channels per plant). At this stage, the monitoring of optical properties of the plants was started. We denote this point in time as t_0. Simultaneously, the plants were subjected to the nutrient deficiency by the exclusion of given salts (3 plants per treatment/ 9 channels per treatment).

The groups of plants were respectively subjected to: nitrogen deficiency [-N], phosphorus deficiency [–P],

manganese deficiency [–Mg], iron deficiency [–Fe], nutrient starvation treatment 'exhausted plants' [–E] (we didn't change nutrient solutions during optical measurements) and the complete nutrient solution – control [C]. In –N treatment the $Ca(NO_3)_2$ was replaced with the adequate concentration of $CaCl_2$. The solutions were continuously aerated and changed every 3 days (excluding E treatment) Table 1.

Starting from $t_{0,}$ the leaf optical transmittance (red light 665 nm) for all channels was recorded in 15 min intervals, for the duration of following 9 days (Figure 4). For each plant, we selected young leafs (usually the third leaf from above of the plant) for the recording.

Determination of photosystem II efficiency

At the end of the experiment, the efficiency of photosystem II was estimated by chlorophyll fluorescence parameters which indicate if there is an influence of environmental stress factors on the photosynthesis. The parameters: minimum (*Fo*) and maximum (*Fm*) fluorescence, following the 30 min period of dark adaptation, and maximum quantum yield of primary PSII photochemistry (photosynthetic efficiency) (*Fo-Fm*)/Fm (*Fv/Fm*), were used to determine the effects of nutrient deficiency stress on photosynthetic apertures. Measurements were taken with the portable plant-stress meter (FluorPenFP 110). Chlorophyll was excited by the actinic light pulse (2–5 s in duration), with the photon flux density of 200 and 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Measurements were taken 4 h after photoperiod in chamber was started. Eight plants were analysed by measuring parameters in 9 channels per three treated plants, on the completely developed 3rd and 2nd leaf from the top.

Determination of photosynthetic pigments content

Photosynthetic pigments content was determined spectrophotometrically in acetone pigment extracts of leaves (younger, fully developed leaves, from the top). Leaf fresh weigh (*FW*) of 1 g was homogenised in10 mL of acetone. To prevent acidification of the solution, a small amount of $MgCO_3$ was added. After homogenisation the content was filtered through a glass filter. Pigment extracts were quantitatively transferred to 25 mL volumetric flasks and diluted up with acetone. Absorptions of the extracts and blanks were read on the spectrophotometer (Thermo, Type evaluation 300 UV-Vis Spectrophotometer) at wavelengths of 662, 644, and 440 nm. Concentration of chlorophyll a, chlorophyll b and total carotenoids were calculated according to Wettstein formula and expressed inmg$\cdot g^{-1}$(*FW*).

Table 1. Components of the nutrient solutions.

Salt (macronutrients)	Concentration in plant growth solution before treatments (mM)	Salt concentration in –N treatment (mM)	Salt concentration in –Mg treatment (mM)	Salt concentration in –P treatment (mM)	Salt concentration in –Fe treatment (mM)
K_2SO_4	0.35	0.35	0.35	0.35	0.35
KCl	0.05	0.05	0.05	0.05	0.05
$Ca(NO_3)_2$	1.00	–	1.00	1.00	1.00
$MgSO_4$	0.25	0.25	–	0.25	0.25
KH_2PO_4	0.05	0.05	0.05	–	0.05
$CaCl_2$	–	0.5	–	–	–
Salt (micronutrients)	Concentration in plant growth solution before treatments (µM)	Salt concentration in –N treatment (µM)	Salt concentration in –Mg treatment (µM)	Salt concentration in –P treatment (µM)	Salt concentration in –Fe treatment (µM)
$Fe^{III}EDTA$	25.000	25.000	25.000	25.000	–
H_3BO_3	5.000	5.000	5.000	5.000	5.000
$MnSO_4$	0.250	0.250	0.250	0.250	0.250
$ZnSO_4$	0.2500	0.250	0.250	0.250	0.250
$CuSO_4$	0.100	0.100	0.100	0.100	0.100
$(NH_4)_6Mo_7O_{24}$	0.005	0.005	0.005	0.005	0.005

Figure 4. *Ocimum basilicum* plants.

Shoot and root dray weight determination

After the treatments, the plants were dried at 70°C to constant weight. Shoot and root dry weight (DW) per plant was measured (g) and root/shoot ratio calculated.

Statistical analysis

Data were subjected to analysis of variance and ANOVA-Turkey's HSD post hoc test was applied using the statistical software Statistica 6 (StatSoft, Inc., Tulsa, OK, USA). Mean and standard errors (SE) for the estimated parameters were determined, and the analysis of variance was applied. Fisher's LSD test was used to compare the arithmetic means of the groups.

Results

Figure 5(a–e) shows percent variations of the coefficients of transmission as a function of time for the control group compared to the other 5 groups. The time interval covered by graphics was 9 days, started from 1st day after the first nutrition solution change. For a given group of channels, measurements were performed by the sequential firing of each channel. The duration of a single sequence used for the measurements was ∼90 s. The sequences were repeated every 15 min for the duration of the experiment (Kasalica et al. 2021).

All six groups exhibit clearly defined day-night Circadian rhythms with modifications in variations of the coefficients of transmission. We have analysed the relative variations of the signals values. Difference in coefficients of transmission ratio depends on the leaf thickness and leaf angle with respect to the optical fibre.

Circadian rhythms had the obvious periodicity, which was characterised with two functions: the daily local minimum (linear) and the daily local maximum (trigonometric periodic function with clearly amplitude modulation). The overall trend of transmission was slow changing function (averaged over one day period) for all 5 groups. Overall characteristics of the graphic trend of the optical transmission were changed. We discussed about the variation of the coefficients of

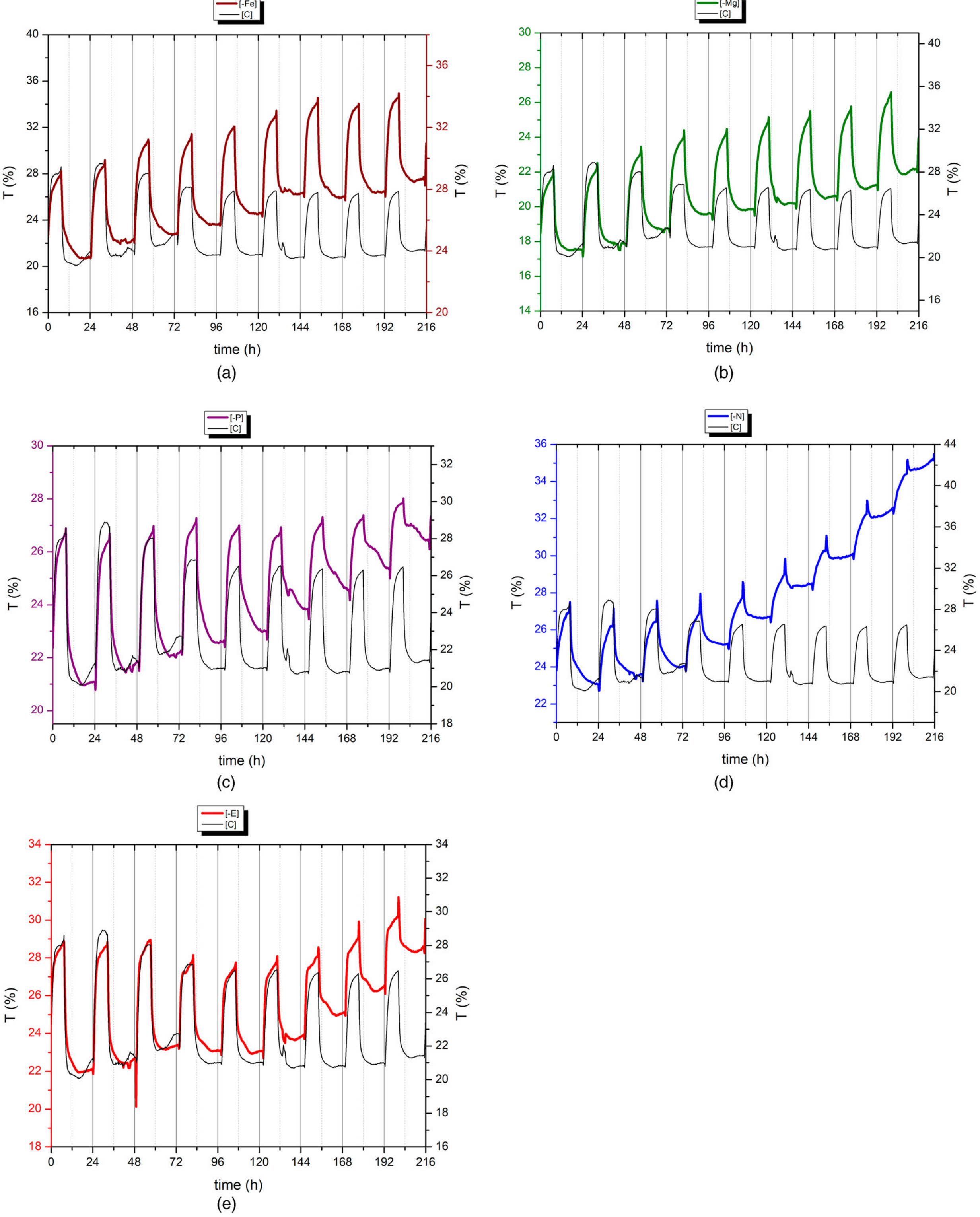


Figure 5. Variation of transmittance T (%) as a function of time in days (started from midnight). (a) Group I (control group) – black, and group II [–Fe] deficiency – vine red. (b) Group I (control group) – black, and group III [–Mg] deficiency – green. (c) Group I (control group) – black, and group IV [–P] deficiency – purple. (d) Group I (control group) – black, and group V [–N] deficiency – blue. (e) Group I (control group) – black, and group VI [–E] (exhausted) – red.

transmission in percentage change (the values of the coefficients of transmission of the 1st day in comparison to the values of the 9th day).

The control group exhibits flat slow function with constant values of daily local minimum (called 'base line'), and daily local maximum with constant day-night amplitude.

Table 2. Photosynthetic pigment content: mean values and standard errors.

Group of plants/treatments	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a + b (mg/g)	Carotenoids (mg/g)
[C] (control)	(0.58 ± 0.03)a	(0.24 ± 0.03)a	(0.81 ± 0.06)a	(0.188 ± 0.004)a
[–Fe]	(0.44 ± 0.08)ab	(0.18 ± 0.03)ab	(0.62 ± 0.11)ab	(0.17 ± 0.02)a
[–Mg]	(0.29 ± 0.08)bc	(0.12 ± 0.03)bc	(0.40 ± 0.11)bc	(0.13 ± 0.03)ab
[–P]	(0.47 ± 0.01)ab	(0.175 ± 0.008)ab	(0.645 ± 0.012)ab	(0.176 ± 0.004)a
[–N]	(0.14 ± 0.01)c	(0.065 ± 0.003)c	(0.206 ± 0.013)c	(0.087 ± 0.004)b
[–E] (exhausted)	(0.32 ± 0.03)bc	(0.141 ± 0.007)bc	(0.46 ± 0.04)bc	(0.166 ± 0.008)a

Note: Means and standard errors followed by the same letter within columns are not statistically significant ($p < 0.05$)

The amplitude range was from 20% (during the day) to 28% during the night.

Nine days after the treatment, the graph of the control group [C] exits the constant values of daily local minimum and constant amplitude values of the daily local maximum.

Figure 5(a) shows percent variations of the coefficients of transmission as a function of time for the second group [–Fe] in comparison to the [C]. Third day after the first nutrition solution change, the daily local minimum function of the Fe deficiency group indicates a slow changing component with the linear increase. Nine days after the treatment, the amplitude values of the daily local maximum coefficients of transmission were constant, but the values of the local minimum increased by 16% compared to the first day.

The third group [–Mg] was compared to [C] (Figure 5 (b)). Third day after the treatment, the daily local minimum has shown a slow changing linear increase compared to the base line. Nine days after the

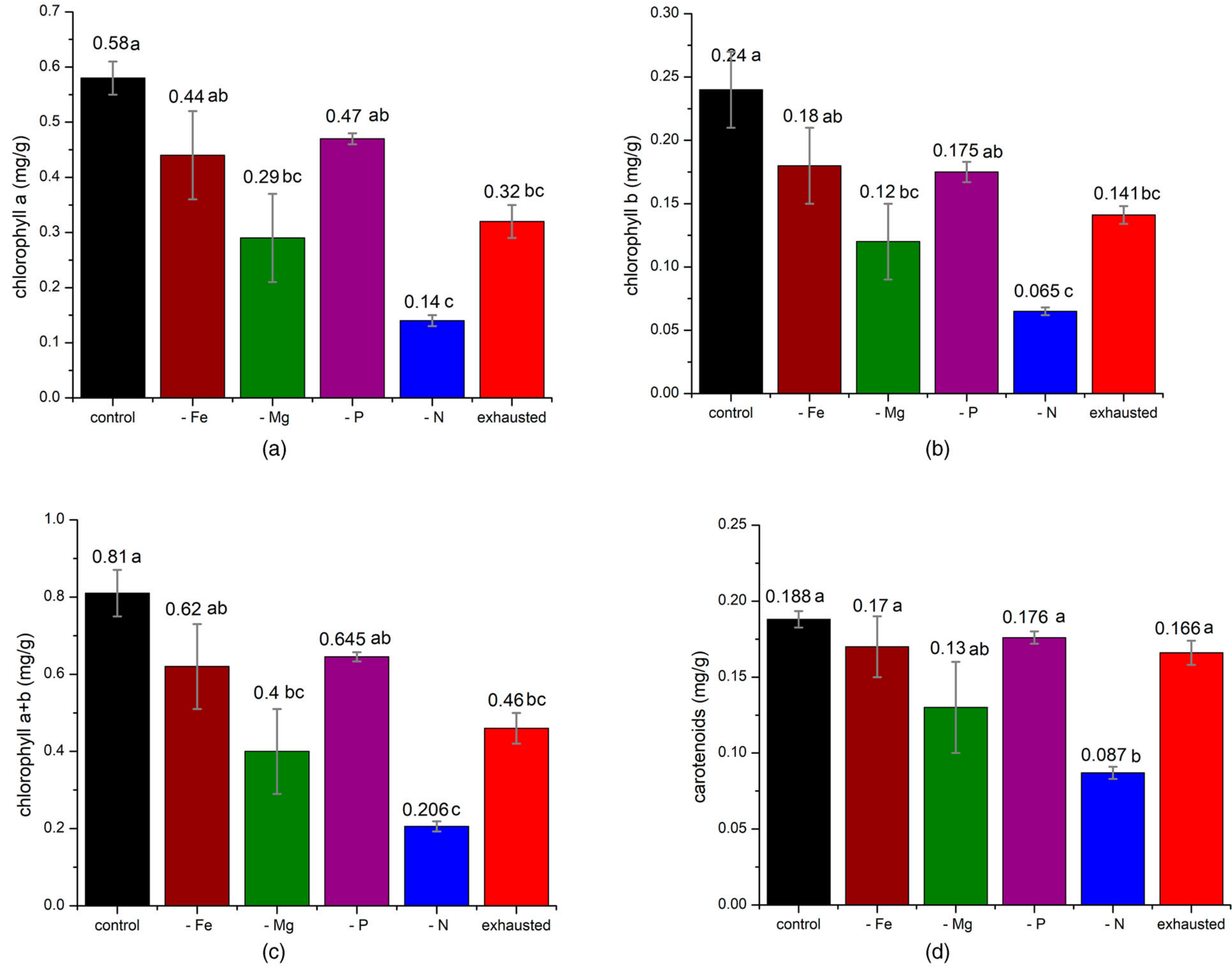


Figure 6. Photosynthetic pigment content: mean values and standard errors. The letters in mean value indicate statistically significant difference in pigment concentration compared to control at $P < 0.05$. (a) Chlorophyll a + b content: mean values and standard errors. (b) Chlorophyll a content: mean values and standard errors. (c) Chlorophyll b content: mean values and standard errors. (d) Carotenoids content: mean values and standard errors.

Table 3. Dry weight of roots and shoots: mean values and standard errors.

Group of plants/treatments	DW ± ΔDW [g] shoot	DW ± ΔDW [g] root
[C] (control)	(2.7 ± 0.5)a	(0.8 ± 0.2)a
[–Fe]	(2.79 ± 0.03)ab	(0.75 ± 0.04)a
[–Mg]	(2.82 ± 0.08)a	(0.52 ± 0.03)a
[–P]	(2.2 ± 0.4)ab	(0.70 ± 0.06)a
[–N]	(1.4 ± 0.2)b	(0.53 ± 0.12)a
[–E] (exhausted)	(1.5 ± 0.3)ab	(0.6 ± 0.3)a

Note: Means and standard errors followed by the same letter within columns are not statistically significant ($p < 0.05$)

treatment, the amplitude values of the daily local maximum were constant, but the values of the local minimum increased by 20% compared to the first day.

Figure 5(c) shows variations of the coefficients of transmission as a function of time for the 4th group [–P] in comparison to the [C]. The four days after the treatment, the daily local minimum has showed a slow changing linear increase. On the other hand, five days after the treatment, the daily local maximum amplitude decreased by 1.5 times. Nine days after the treatment the daily local maximum amplitude decreased triple times and the daily local minimum increased by 23% compared to the first day.

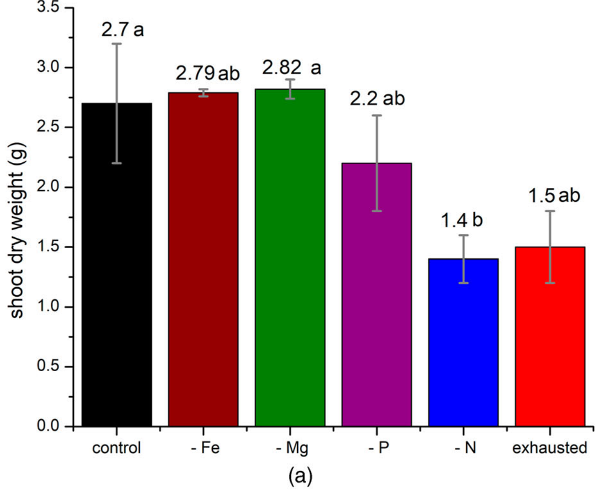
The fifth group [–N] was compared to the [C] and the results are presented in Figure 5(d). The second day after the treatment, the daily local minimum has shown a slow changing linear increase, but four days after the treatment, the daily local minimum has showed a fast changing function increase with a noticeable change of the day local maximum amplitude. Nine days after the treatment the range of values of the daily local maximum amplitude decreased four times and local minimum increased 32% compared to the first day.

The sixth group [–E] (nutrient starvation treatment) was also compared to [–C] and the results are shown in Figure 5(e). Third day after the treatment, the circadian function has shown a slow changing local daily linear increase with a noticeable change of the daily local maximum amplitude. Nine days after the treatment the range of values of the local daily linear minimum coefficients of transmission increased by 21%, and function amplitude decreased double times compared to the first day.

In our experiment the concentrations of chlorophyll *a* and chlorophyll *b* particularly decreased in [–N], [–Mg] and [–P] treatments, 9 d after the specific nutrient starvation. This is a clear indication of an observed nutritional stress. The [–E] and [–Fe] treatments didn't show statistically significant difference when compared to the control plants (Table 2 and Figure 6(a–d)). The concentration of carotenoids significantly decreased only in leaves from [–N] and [–Mg] groups (Table 2 and Figure 6(d)).

The dry weight (DW) of shoots significantly decreased in [–N] and [–E] groups. The dry weight (DW) of roots significantly decreased in [–N], [–Mg] and [–E] groups. In all other treatments, only a slight reduction of DW was observed (Table 3, Figure 7(a,b)).

DW decrease was observed only in leaves with severe chlorosis. In our experiment, the fluorescence parameters: minimum (*Fo*) and maximum (*Fm*) and the photosynthetic efficiency (*Fv/Fm*) (Table 4, Figure 8(a–c)) indicate a nutritional stress in [–N], [–Mg] and [–E] treatments (Figure 5(b)). In these treatments, the value was lowest in 'exhausted plants' (0.39) indicating very low activity of photosystem II of dark-adapted



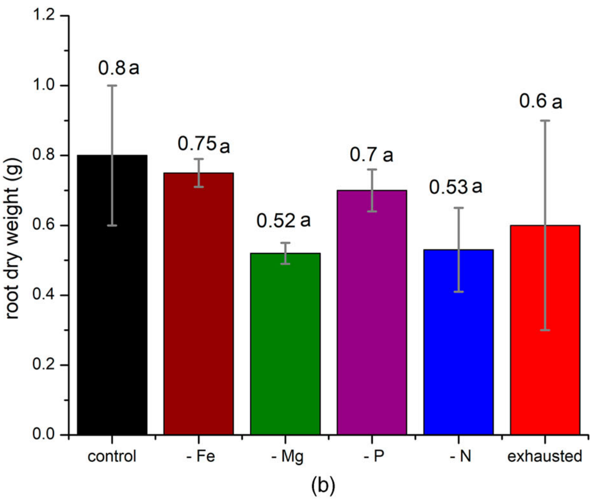


Figure 7. Dry weight of roots and shoots: mean values and standard errors. The letters in mean value indicate statistically significant difference in pigment concentration compared to control at $P < 0.05$. (a) Dry weight of shoots: mean values and standard errors. (b) Dry weight of roots: mean values and standard errors.

Table 4. Parameter *Fo*, *Fm* and *Fv/Fm*: mean values and standard errors.

Group of plants/ treatments	$(Fo \pm \Delta Fo)\cdot 10^3$	$(Fm \pm \Delta Fm)\cdot 10^4$	$(\frac{Fv}{Fm} \pm \Delta\frac{Fv}{Fm})$
[C] (control)	(12.0 ± 0.7)bc	(63.0 ± 0.2)a	(0.812 ± 0.006)a
[–Fe]	(10.0 ± 0.5)c	(55.0 ± 0.6)a	(0.818 ± 0.014)a
[–Mg]	(17 ± 3)bc	(51.0 ± 0.4)a	(0.67 ± 0.04)a
[–P]	(12.0 ± 0.6)bc	(61.0 ± 0.3)a	(0.80 ± 0.01)a
[–N]	(23 ± 3)ab	(80.0 ± 0.6)a	(0.72 ± 0.03)a
[–E] (exhausted)	(32 ± 6)a	(64.0 ± 1.6)a	(0.40 ± 0.11)b

leaves. Treatments [–P] and [–Fe] were in the same homogenous group with the control (*Fv/Fm* from 0.80 to 0.82) indicating that the activity of photosystem II wasn't significantly affected (Figure 8(b)).

Discussion

To our knowledge, this is the first published long-term time dependence of the optical transmission (Circadian rhythms) of the assimilation organs (leaves) (Kasalica et al. 2021), of the plants which were exposed to the nutritional stress during the plant growth.

Tissue nutrient analysis implies destructive procedure needed to assess nutrient status. It is usually based on chemical and histochemical methods (concentration analysis) or biochemical for the assessment of enzyme activity.

On the other hand, the indirect nondestructive methods, based on chlorophyll-a florescence, are often not the best choice because the nutrient deficiency is not always related to the decrease of photosystem II activity. This is well documented for the cases of iron and phosphorous deficiency. In order to demonstrate that P deficiency in plants is highly reversible and that disruptions in electron transport can easily be regained, Carstensen et al. (2018) infiltrated P- deficient leaf segments with Pi containing solution for short period of time and showed that in the I-step transients in P-deficient plants was highly reversible and quantum efficiency of PSII (*Fv/Fm*) was unable to discriminate between the treatments because all transients reached values close to maximum fluorescence (0.83). This finding is compatible with our results shown in Table 4 and Figure 8(c). The values of the photosynthetic

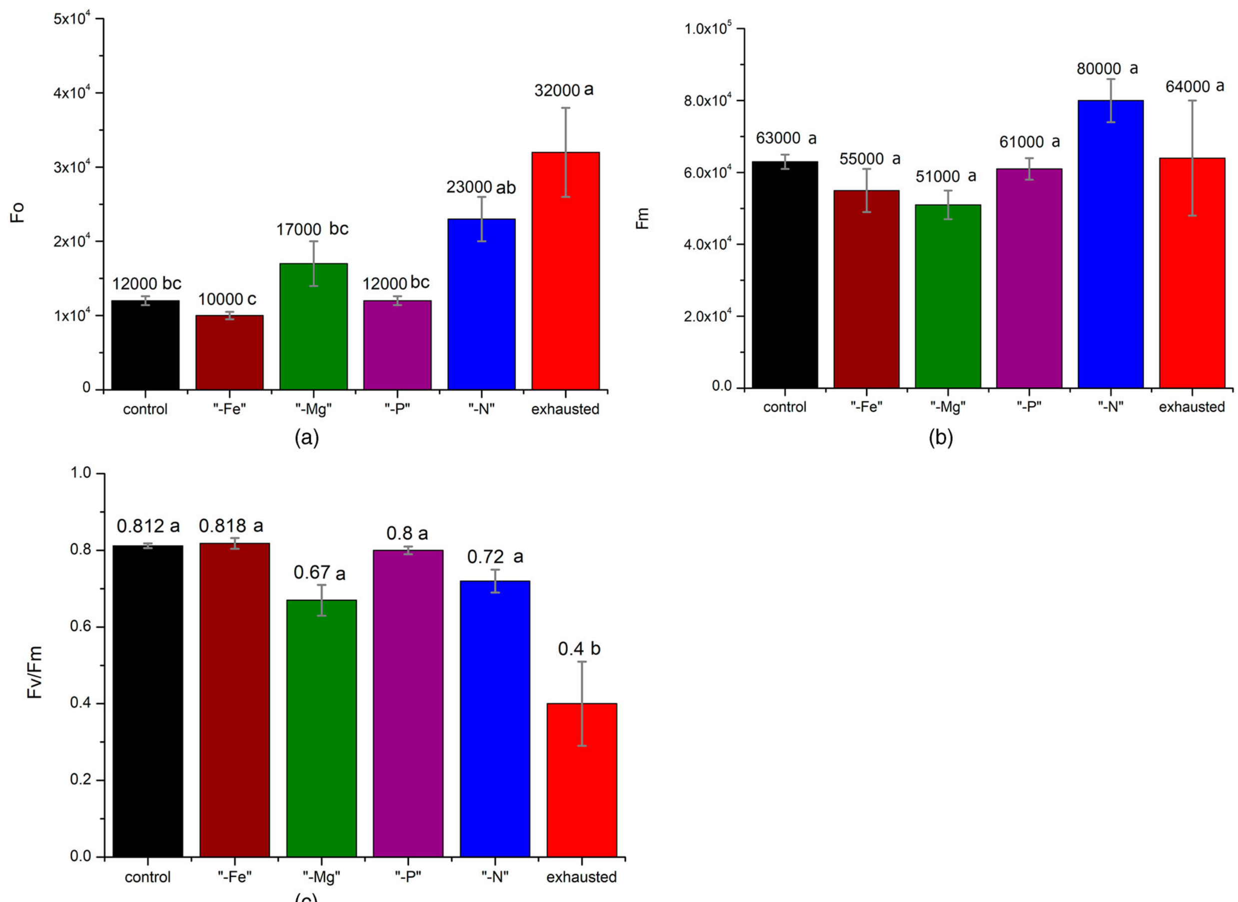


Figure 8. Parameter *Fo*, *Fm* and *Fv/Fm*: mean values and standard errors. The letters in mean value indicate statistically significant difference in pigment concentration compared to control at $P < 0.05$. (a) Parameter *Fo*: mean values and standard errors. (b) Parameter *Fm*: mean values and standard errors. (c) Parameter *Fv/Fm*: mean values and standard errors.

parameters of PSII for P-deficient group did not show decrease in PSII efficiency (*Fv/Fm:* 0.80) compared to the control group (*Fv/Fm*: 0.81). However, our results in Figure 5(c) showed a slow changing linear increase, suggesting the plant stress.

In the research of Morales et al. (2000), Fe deficient leaves did not show decrease in PSII efficiency after the dark adaptation. This is in consistence with our results shown in Table 4 and Figure 8(c) (*Fv/Fm*: 0.818) compared to the control group (*Fv/Fm*: 0.812). On the other hand, Figure 5(a) suggested the plant stress of the group which was subjected to Fe deficiency by excluding Fe.

A recent work on the deficiency treatments response for the minerals N, P and Mg with PSII decreased is presented in work of the M. Ohnishi (Ohnishi et al. 2021). As it was presented, it is possible to determine connection between N-deficient plants which indicate the lowered electron-sink activity probably because of the lack of an N source for biosynthesis of Rubisco (John Andrews and Lorimer 1987; Tränkner et al. 2018), which is the largest destination of fixed nitrogen (Makino and Osmond 1991).

Moreover, Ohnishi et al. (2021) showed lowered *Fv/Fm* for Mg-deficient plants. The same phenomenon is observed in our results, which show that the efficiency of PSII of dark-adapted leaves of *Ocimum basilicum* are decreasing in plants under nitrogen and magnesium short-term nutritional stress (*Fv/Fm*: 0.72 and 0.67 respectively) compared to the control group (*Fv/Fm*: 0.81) as it is shown in Figure 8(c), Table 4, and Figure 5 (b,d), respectively.

In the research of Jaghdani et al. (2021), in experiment with spinach, the shoot DW was not significantly reduced whereas root DW was substantially affected under Mg deficiency. These results are consistent with our work (Table 3, Figure 7(a,b)).

In addition, the onset of the observed optical transmission changes happens well before the plants' stress could be observed with the more traditional methods such as chlorophyll fluorescence parameters and chlorophyll content (Shah et al. 2017). Syed Shah et al. (2017) conducted a greenhouse experiment using SPAD-502 measurements on wheat (*Triticum aestivium* L.), fast and non-destructive approach to determine relative values of chlorophyll content in leaves in various amounts of salinity and fertiliser, also, reduction of the chlorophyll content has been observed in Fe- deficient plants (Ohnishi et al. 2021).

Our results of the photosynthetic pigment content in all of the plants deficiency treatments significantly decreased comparing to the control group (Table 2, Figure 6(a,d)). This is with line with findings by Shah et al. (2017) and Ohnishi et al. (2021).

Our data is also related to findings by Nenova 2008 on other plant species. Nenova (2008) did series of experiments with hydroponically grown pea plants. They supplied them with different amounts of iron (Fe) ranging from complete deficiency to toxicity and confirmed that chlorophyll concentration is very sensitive to Fe supply. Significant changes were observed in chlorophyll fluorescence in strongly chlorotic plants only. The essential mineral deficiencies had different effects on the chlorophyll content (Table 2, Figure 6(a–d)). The Fe, Mg, P, N, and E deficiencies significantly decreased the chlorophyll content when compared to the control plants ($p < 0.05$).

Comparing photosynthetic pigment content, the shoot and root DW and sensitivity of quantum efficiency of PSII (*Fv/Fm*) to a new method based on transmittance measurement at 665 nm in all of the nutrient deficiency treatments, we concluded that a new method was more sensitive in capturing activity decrease of the plants exposed to nutritional stress. This fact allows for the early intervention and the prevention of malnutrition caused damages in hydroponically grown crops. The additional advantage of the described method is the fact that it is nondestructive.

Disclosure statement

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Notes on contributors

Katarina M. Miletic is a doctoral candidate, research assistant of Faculty of Physics, University of Belgrade. Her research fields are biophysics, applied physics and metrology.

Danijela M. Djunisijevic-Bojovic is an associate professor at the Faculty of Forestry UB with research experience related to the influence of abiotic factors on physiological processes in plants, with a focus on adaptive mechanisms of plants to nutritional stress and heavy metals toxicity.

Becko V. Kasalica is a full professor at Faculty of Physics, University of Belgrade. His research areas are applied physics and metrology, material science, spectroscopy and optical measurements.

Marijana Milutinovic is a Ph.D. student at the Faculty of Forestry UB. Her research is focused on the adaptive mechanisms of invasive plants to phosphorus deficiency.

Marija M. Petkovic-Benazzouz is a research associate at Faculty of Physics, University of Belgrade. Her areas of scientific interest are applied physics, material science and molecular modelling.

Slobodan D. Milanovic is an associate professor at the Department of Forestry, University of Belgrade Faculty of Forestry. His research areas are forest protection, interactions between plant pathogens and herbivores mediated by trees, and forest fire modelling.

Ivan D. Belca is a full professor and dean at the Faculty of Physics. His research fields are solid state physics, applied physics-metrology.

Mirjana Z. Sarvan is an associate professor at Faculty of Physics, University of Belgrade. Her research fields are applied physics and metrology, spectroscopy, optical measurements and biophysics.

Dejan A. Jeremic is a research associate at Innovation Center of Belgrade, Department of General and Inorganic chemistry. His areas of scientific interest are applied chemistry, bioinorganic chemistry, inorganic material chemistry and chemical photobiology.

ORCID

Katarina M. Miletic http://orcid.org/0000-0001-9477-6640
Danijela M. Djunisijevic-Bojovic http://orcid.org/0000-0002-4926-0319
Becko V. Kasalica http://orcid.org/0000-0002-8300-6234
Marijana Milutinovic http://orcid.org/0000-0002-4513-1851
Marija M. Petkovic-Benazzouz http://orcid.org/0000-0003-3526-2054
Slobodan D. Milanovic http://orcid.org/0000-0002-8260-999X
Ivan D. Belca http://orcid.org/0000-0001-6124-5333
Dejan A. Jeremic http://orcid.org/0000-0001-9950-0803

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