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Effect of Hydrogen Peroxide on Pigment Composition and Chlorophyll Fluorescence in Basil (Ocimum Basilicum L.) Leaves

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Abstract **- Basil is one of the most famous herbs, widely used fresh, and for therapeutic and pharmaceutical purposes. Basil has recently been shown to rank first among spices and herb crops regarding xanthophyll-carotenoids, which are associated with a reduced risk of cancer and age-related eye diseases. Given the widespread use of the herb basil in nutrition and herbal medicine, the contents of its leaves are of obvious interest. However, some studies say that water enriched with hydrogen peroxide helps more effectively saturate plant cells with oxygen molecules, activates the growth processes of all parts of seedlings, and increases the resistance of seedlings to various unfavorable factors, including dangerous fungal and bacterial diseases (late blight, downy mildew, root rot, powdery mildew, bacteriosis). However, in high concentrations, hydrogen peroxide also can have a phytotoxic effect on plants. The research goal for the current study was to determine the application of hydrogen peroxide (H_2O_2) on photosynthetic processes (the content of photosynthetic pigments, the parameters of the chlorophyll a fluorescence) in sweet basil (*Ocimum basilicum* L. cv. "Genovese"). The experiment was carried out under controlled conditions with three replications. Four treatments of H_2O_2 were tested: 0.0, 0.1, 0.5, 1.0 and 3.0%. Both photosynthetic pigments and parameters of the chlorophyll a fluorescence in basil leaves were quantified and compared with the control. The plants were cultivated in an environmental climatic chamber with conditions close to real natural conditions (temperature: 24-30°C, humidity: 70 – 80%, photoperiodism: day/night (8/16), illumination: 10 000 lux). The present results revealed the changes in chlorophyll fluorescence parameters in basil under different concentrations of H_2O_2 (0.1, 0.5, 1.0 3.0%). In addition, changes in photosynthetic pigment composition have also been noticed depending on the concentrations of H_2O_2 in the water before watering the plants. However, it is necessary to continue studying the effect of H_2O_2 on the whole plant in future research**

Keywords - basil, carotenoids, chlorophylls, hydrogen peroxide (H_2O_2), Ocimum basilicum L

I. INTRODUCTION

Basil (*Ocimum basilicum* L.) is one of the world's most popular aromatic, medicinal, and spice plants of the *Lamiaceae* family. Basil grows in tropical and subtropical regions from Central Africa to temperate Southeast Asia and is considered an annual plant [3]. Basil is a plant rich in various biologically active compounds such as flavonoids, phenolic acids, carotenoids, and chlorophylls. Various phenolic acids have been identified in basil leaves: rosmarinic, gallic, caffeic, chlorogenic, hydroxybenzoic, vanillic, ferulic, and transcinnamic [29] with strong antioxidant activities. In the group of flavonoids, rutin, naringin, naringenin, and quercetin were found [6]. Green and purple basil leaves contain a lot of pigments such as chlorophylls, protective carotenoids, and anthocyanins that are active in the process of

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photosynthesis [9]. Basil leaves contain many essential oils that are used to treat some ailments, such as headaches and colds, as well as severe diseases like diarrhea and kidney failure, as well as some of the aromatic herbs already studied [4], [23], [28]. The most important compounds of the essential oils are eucalyptol, linalool, and methyl chavicol. Minor compounds are neral, 1,8-cineol, and myrcene [13]. Such a high concentration of various active substances gives basil phenomenal medicinal, pharmaceutical, and health properties, including anti-inflammatory, antioxidant, and antibacterial activities. The active substances of this plant are used to treat flatulence, strengthen the digestive system, and relieve pain and fatigue [23]. Studies have also revealed other medicinal properties of basil, such as lowering blood pressure and fever, lowering blood glucose and cholesterol levels, suppressing muscle spasms and inflammation, and enhancing the body's natural activity [7]. Depending on the basil variety, environmental conditions, and harvest time, the exact concentration and composition of these active compounds may vary.

Hydrogen peroxide (H_2O_2) at high concentrations causes cellular damage, whereas, at low or moderate concentrations, it acts as a second messenger in intracellular signalling cascades, triggering various metabolic reactions and enhancing the ability of plants to withstand adverse conditions [2], [12]. However, since H_2O_2 is considered a reactive oxygen species (ROS), its improper use can cause negative effects and damage cell membranes, leading to oxidative damage and programmed cell death [24].

II. MATERIALS AND METHODS

A. Plant material and growth conditions

Sweet basil (*Ocimum basilicum* L. cv. "Genovese") was evaluated for leaf tissue carotenoid and chlorophyll pigments. Basil seeds (country of origin: Latvia) were cultivated in an environmental climatic chamber (MLR-351H, Versatile Environmental test Chamber, Sanyo, Japan) close to real natural conditions (temperature: 24-30°C, humidity: 70 – 80%, photoperiodism: day/night (8/16), illumination: 10 000 lx). Four treatments of H_2O_2 (Chempur Hydrogen Peroxide 30% pure p.a., Poland) were tested: 0.1, 0.5, 1.0, and 3.0% and control (absence of H_2O_2). Seeds were watered to maintain the required humidity of the growing medium.

B. Pot preparation and soil of the experiment

Basil seeds (cv. "Genovese") were planted in polyvinyl chloride (PVC) pots with a diameter of 11 cm, a height of 12 cm, and a soil volume of 1 L. Each pot was filled with commercial substrate (Versatile flower and vegetable substrate: Garden Guru, Latvia). The physical and chemical characteristics of the potting soil: pH_{KCl} 5.5-6.5, electrical conductivity (EC): 0.1-0.6 mS/cm, calcium (Ca) – 1.1%, magnesium (Mg) – 0.07%, moisture – max. 60%, substrate fraction 0-20 mm. Three seeds preselected by size and quality were planted at a depth of 2 cm in each pot, and after the seedlings' germination and establishment, one plant was left in each pot, and the rest were thinned out. When the basil plants grew to 15 cm after 4 weeks, they were used for experimental purposes. For experimental purposes, only basil leaves were used

C. Determination of photosynthetic pigments

The quantification of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (Car) content were evaluated through solvent extraction (80% acetone), according to the methodology [20]. 200 mg sample of examined plant material was extracted with cold 80% acetone (-20°C) in a pre-chilled mortar, and then, magnesium carbonate was added. After complete extraction, samples were centrifuged twice (7000 rpm, 4°C, 10 min), and the volume was brought to 10 mL with cold acetone. The samples were thoroughly mixed and the absorbance of the test samples was measured at the following wavelengths: 663 nm, 646 nm, and 470 nm (the spectrophotometer was calibrated using cold 80% acetone). The values obtained were used to calculate the results, and these were expressed in (mg/L) of pigment in fresh leaf tissue

D. Chlorophyll fluorescence measurements

Chlorophyll fluorescence measurements were carried out at room temperature (20 ± 1°C) using a modulated OS- 30 Chlorophyll fluorimeter (Opti Sciences, US) with a clip-on holder for plant leaves. The dark-adapted basil plants were used for the measurement of minimal (F_0) and maximal (F_m) chlorophyll fluorescence. The basil plant was exposed to bright light for 60 seconds (fluorescent light was used as the light source: photosynthetically active photon flux: 150 µmol m^{-2} s^{-1}, luminance: 12 400 Lx. For the dark-adapted F_v/F_m test, the following sampling intervals were used: 30 min; 60 min; 90 min; 120 min, and 24 h after bright light exposure for each concentration as recovery period [21]. The PS2 photochemical efficiency or the ratio of variable to maximum fluorescence (F_v/F_m) of dark-adapted plants was calculated automatically according to F_o and F_m and measured by the formula: [$F_v/F_m = (F_m-F_o)/F_m$].

D. Statistical analysis

The results obtained were statistically evaluated using Microsoft Excel Ver. 14.0.7214.5000 software. Each experiment was done in three replicates ± standard error (SE).

III. RESULTS AND DISCUSSION

The chlorophylls (Chl) and carotenoids (Car) are integral components of thylakoid membranes and act as accessory pigments in light trapping as photoprotective agents, dissipating rapidly absorbed excess light [17]. Measured Chl *a* and Chl *b* concentrations in basil ranged from 21.94 to 24.33 mg/L FW and 6.70 to 8.29 mg/L FW, respectively, as depicted in (Fig. 1).

Compared to Car pigments, basil had a much higher concentration of Chl pigments in leaf tissues. In leaves of

basil seedlings Chl *a* and Chl *b* concentrations were enchased by applying H_2O_2 treatment. In plants treated with H_2O_2 (0.1%), there was an increase in Chl *a* concentration (24.33 mg/L FW), compared to the control (22.49 mg/L) and to plants treated with 1% and 3% H_2O_2 (23.32 and 24.07 mg/L FW). However, in plants treated with H_2O_2 (0.5%), there was a decline in Chl *a* content (21.94 mg/L FW). The obtained results showed that Chl *b* measured in plants treated with 0.1%, 1% and 3% H_2O_2 was 7.91, 7.63, and 8.29 mg/L FW, whereas, plants treated with 0.5% H_2O_2 Chl $_b$ was 6.70 mg/L FW. The values of the concentrations of photosynthetic pigments of the examined plant varied greatly depending on the applied treatment.

By analysing the obtained results for the concentration of photosynthetic pigments treatment with H_2O_2 had a slight increasing impact. This increase can be partly explained by the role of H2O2 in protecting the chloroplast ultrastructure and thus increasing the efficiency of the photosynthetic apparatus [23]. Some authors associate the signalling role of H_2O_2 with the protective function related to maintaining the ultrastructure of chloroplasts. Previous studies have observed a significant increase in Chl concentration in plants treated with H_2O_2 [15], [25]. On the other hand, Mani et al. demonstrated that applying H_2O_2 at a low concentration (20mM) decreased Chl content in *Solanum tuberosum* L. [16].

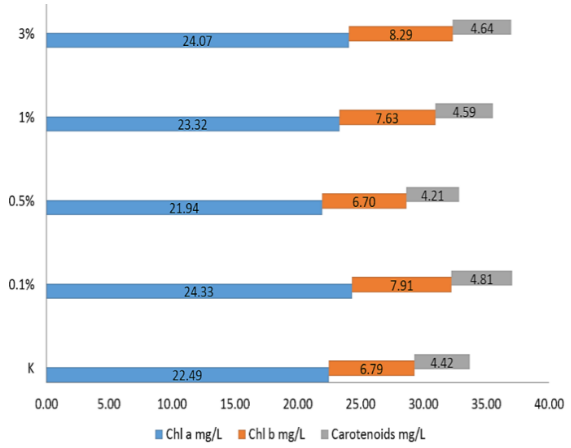

Fig. 1. Chlorophyll *a*, Chlorophyll *b*, and carotenoids content changes in leaves of sweet basil (*Ocimum basilicum* L. cv. "Genovese") under the influence of H_2O_2

Carotenoids act in the process of energy dissipation in the form of heat, reducing the deleterious effects of stress on plants [27]. The highest (4.81 mg/L FW) concentration of total carotenoids was measured after the treatment with H_2O_2 (0.1%) compared to the control treatment (Fig. 1). In the present experiment, compared to the control treatment (4.42 mg/L FW), the 1% and 3% H_2O_2 concentrations increased Car concentrations (4.59 mg/L FW and 4.64 mg/L, respectively) (Fig. 1). An increase in the concentration of carotenoids occurs as a mechanism of photoprotection of plants under stress conditions. In general, Car are compounds connected with Chl synthesis and are isoprenoids that, in addition to helping to capture light, are also directly related to the photoprotection mechanism in plants under stress conditions [25], [26].

A treatment with H_2O_2 leads to a series of physiochemical changes that modify the protoplasmic characteristics of plant cells, improving the physiological activity of embryos and future seedlings [30]. Since H_2O_2 is a multifunctional molecule, at lower concentrations it is involved in the regulation of plant growth, development, and physiological processes [8]. The application of H_2O_2 results in the maintenance of protein structure and regulation of enzymes responsible for protein synthesis [21]. Additionally, H_2O_2 has been shown to stimulate photosynthetic processes by improving gas exchange, Chl content, and protection, and scavenging ROS [19]. These facts were also confirmed by [14], [10].

The maximum photochemical efficiency or quantum yield (F_v/F_m) can be used to estimate the photosystem II (PSII) operational efficiency. It indicates the capacity of absorption of excitation energy by leaves, and it is usually decreasing thereafter because of leaf senescence and a decrease of photosynthetic assimilation [5]. In the control condition, the ratio (F_v/F_m) is generally around 0.8, and it decreases in stressful situations [1], [31]. However, high values of this ratio characterize non-stressed leaves [18].

Concerning plants treated with H_2O_2, the presented results showed that the maximum photochemical efficiency (F_v/F_m) values were similar for different treatments (Fig. 2). They ranged from 0.731 to 0.781. This ratio was high (0.781 and 0.769) when plants were treated with respectively 0.5% and 3% of H_2O_2. Notably, the lowest basil F_v/F_m values were observed treated with 0.1% and 1% of H_2O_2 (0.731 and 0.744 respectively), while the maximum basil F_v/F_m value was recorded at control plants (0.803).

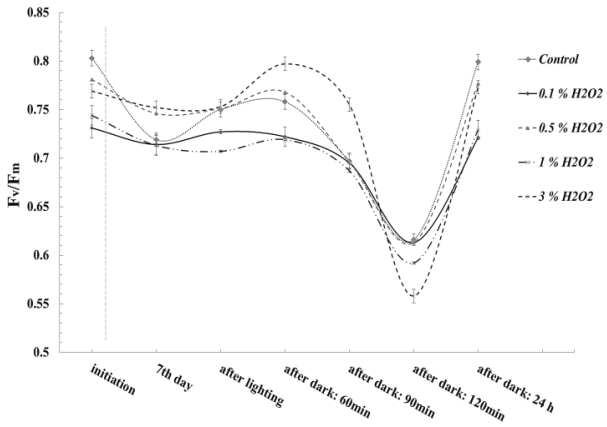

Fig. 2. F_v/F_m parameter changes in sweet basil (*Ocimum basilicum* L. cv. "Genovese") under the different concentrations of H_2O_2.

The photosynthetic efficiency (F_v/F_m), as measured in dark-adapted leaves (after 24 h) treated with H_2O_2, expressed no significant changes in comparison to the control material except for leaves treated with 0.1% and 1% H_2O_2 (0.721 and 0.729, respectively). Thus, the

maximum photochemical efficiency (F_v/F_m) of leaves of plants treated with H_2O_2 depends on the used concentrations. The reduced value of this ratio (F_v/F_m) measured in plants treated with H_2O_2 may be related to the conformational changes in the reaction centre of PSII [11], [22].

IV. CONCLUSIONS

The study demonstrated that hydrogen peroxide (H_2O_2) plays a significant role in the universal regulatory and signalling mechanisms of plant cells. Its impact on plant physiology is clearly evident, particularly under stress conditions. By optimizing environmental parameters, it is possible to modulate the intensity of metabolic processes, enhance nutrient uptake and transport, strengthen immune responses, and improve stress tolerance - ultimately contributing to increased crop productivity.

The data obtained provide a foundation for further investigation into the fundamental properties of H_2O_2 and its functional role in plant development and adaptation. A deeper understanding of the mechanisms by which various factors influence plant cells enables targeted regulation of physiological processes and offers new strategies for enhancing agricultural productivity.

Based on the study's findings, it can be concluded that hydrogen peroxide may act as a signalling molecule, triggering the activation of cellular defense responses - such as the synthesis of antioxidant enzymes - when plants are exposed to adverse environmental conditions.

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