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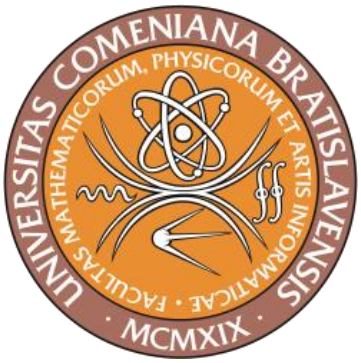

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EFFECT OF PLASMA ACTIVATED WATER ON LETTUCE

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The effect of plasma activate water (PAW) generated by transient spark discharge on lettuce was investigated and compared with the effect of H_2O_2 and/or NO_3^- synthetic solutions. The growth parameters, number and quality of leaves, fresh and dry weight, photosynthetic pigment content, photosynthesis rate and activity of antioxidant enzymes in above-ground parts and roots were measured.

1. Introduction

Cold plasma has potential to be used in various agricultural applications [1]. The majority of the studies deal with direct plasma treatment of seed for germination and growth improvement [2-5]. Besides, indirect plasma treatment, i.e. effect of plasma activated water (PAW) on seeds and plants has recently become of interest [6,7].
The cold plasma generated by the atmospheric pressure air discharges is a source of various reactive oxygen and nitrogen species RONS, namely $^{\bullet}OH$, H_2O_2, NO_2^-, NO_3^- that may dissolve in water and change its chemical composition while producing PAW. The long lived species in PAW (H_2O_2, NO_2^-, NO_3^-) may act as signal molecules in plant metabolism or be a source of nutrients. Encouraged by our previous results on wheat [8], we studied the effect of PAW irrigation on lettuce plants and compared it with effect of H_2O_2 and/or NO_3^- synthetic solutions to understand the role of individual reactive species in PAW.

2. Experimental

The PAW was generated by DC driven self-pulsing transient spark (TS) discharge at atmospheric pressure in air. The TS discharge was produced in a reactor with tap water circulating through the discharge zone. The experimental setup is depicted in Fig 1a. The plasma reactor consisted of high voltage needle and grounded plane inclined electrode in mutual distance ~ 1 cm. The discharge was driven by positive DC power supply (Technix RS20-R-1200) and its electrical characteristics were monitored by high voltage probe (Tektronix P6015A) and Rogowski type current probe (Pearson Electronics 2877) connected to an oscilloscope (Tektronix TDS 1012). The typical amplitude of the applied voltage used in our experiments was U_{app} = 16 kV, amplitude of the breakdown voltage U_{br} = 10 - 13 kV, frequency of the discharge current pulses f ~ 2 - 3 kHz, amplitude of current pulses I_{max} = 6 - 8 A and average discharge power was ~ 6 W. The characteristic voltage and current waveforms of the TS discharge are depicted in Fig. 1b.

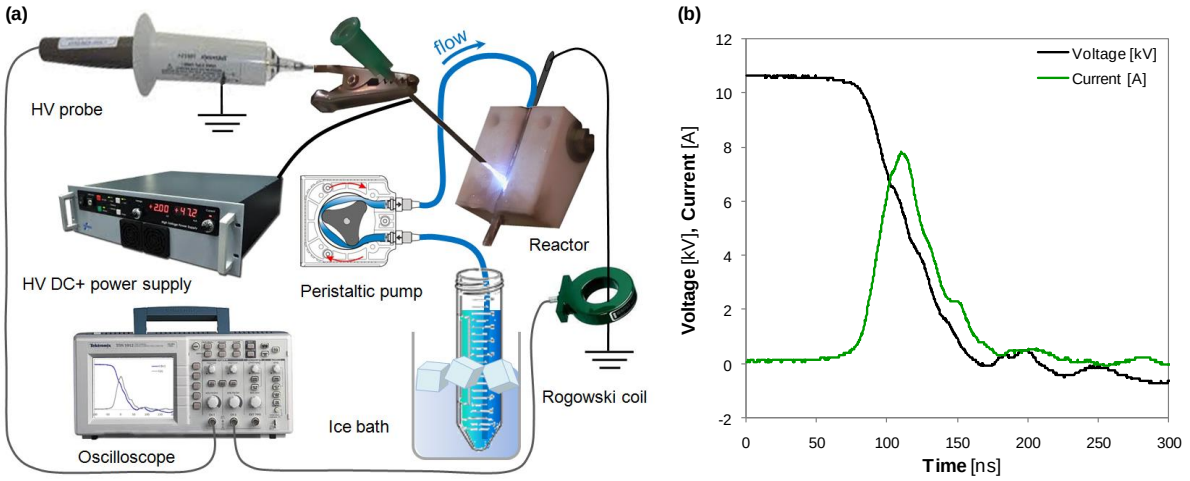

Fig. 1. The scheme of experimental setup (a) and characteristic voltage and current waveforms of self-pulsing transient spark (TS) discharge (b).

The PAW was produced from tap water as it is more physiological for plants than deionized water. Moreover tap water preserves the constant pH during the activation by discharge. The water flow rate through the discharge was 14 mL/min. The activation time was 1 min/mL (i.e. every 1 mL of water was activated for 1 min). The chemical composition of PAW was measured by established UV/Vis spectrophotometric methods to characterize the concentration of hydrogen peroxide H_2O_2, nitrites NO_2^- and nitrates NO_3^-. The H_2O_2 concentration was determined by its reaction with titanyl ions of $TiOSO_4$ [9]. The NO_2^- and NO_3^- concentrations were determined by the commercial kit using Griess reagents (*Cayman chemicals*).

As a model plant was used lettuce (*Lactuca sativa* L. var. capitata cv. Král máje I). The plants were cultivated in pots with soil at controlled conditions: 24/18 °C (light-dark), light intensity 120 $\mu mol.m^{-2}.s^{-1}$ and 12 hours photoperiod. Three weeks old plants were separated to one plant per pot and irrigated with PAW or with H_2O_2 and/or $NaNO_3$ synthetic solutions of various concentrations for 4 weeks (Tab. 1). The control plants were irrigated with tap water only.

Tab. 1. Concentration of H_2O_2 and NO_3^- in solutions used for plants irrigation

Name of solution	**H_2O_2 [mM]**	**NO_3^- [mM]**
Control - tap water	-	~ 0.02
PAW 1 min/mL	~ 0.42 ± 0.12	~ 0.85 ± 0.18
H_2O_2 I	0.4	-
H_2O_2 II	1.0	-
H_2O_2 III	10.0	-
NO_3^- I	-	0.85
NO_3^- II	-	2.0
NO_3^- III	-	20.0
Combination I	0.4	0.85
Combination II	10.0	20.0

The complex evaluation of growth parameters (number and quality of leaves, fresh and dry weight), photosynthetic pigments content, soluble protein content, rate of photosynthesis and activity of antioxidant enzymes of lettuce were performed. We also evaluated the visual appearance of the lettuce. The **fresh weight** of above-ground parts and roots of lettuce were measured separately. After drying at 60 °C packed into aluminum foil, also the **dry weight** were determined separately for above-ground parts and roots. The concentration of **photosynthetic pigments**, i.e. chlorophylls and carotenoids, in leaves were measured according to Lichtenthaler [10]. The measurement of **photosynthesis rate** was performed by infrared analyzator (*CIRAS-2*) based on rate of CO_2 exchange in dependence on light intensity (PAR - photosynthetic active radiation [$\mu mol.m^{-2}.s^{-1}$]). The **activity of antioxidant enzymes**,

namely superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (G-POX) and ascorbate peroxidase (APX) were measured according to standardized assays and normalized to soluble protein content in sample. The data are presented as mean values ± standard deviation. The one-way analysis of variance (ANOVA) and subsequent multiple range test by least significance difference method (LSD) were performed to judge the differences between groups. The different lower case letters represent significant difference at $p < 0.05$.

3. Results

To understand the effect of PAW on lettuce it is necessary to know its composition. In the presented experiments we irrigated the lettuce with 1 min/mL PAW. Immediately after the activation the PAW had pH ~ 7.5 and concentration of RONS were ~ 0.42 mM H_2O_2, ~ 0.38 mM NO_2^- and ~ 0.85 mM NO_3^-. The effect of PAW on lettuce was compared with the effect of H_2O_2 and/or NO_3^- synthetic solutions. Several solutions of the same and also higher concentrations of H_2O_2 and/or NO_3^- than those in the PAW were prepared and used (Tab. 1).

Lettuce plants irrigated with PAW had similar number of leaves, but differed in leaf size compared to control (Fig. 2). Plants irrigated with synthetic solution containing NO_3^- had slightly higher number of green than senescent leaves compared to control. The high concentration of H_2O_2 negatively affected the appearance of plants. However, in combination with high NO_3^- concentration the positive effects of NO_3^- prevailed, as plants with proper nutrition can better handle stress.

Fig. 2. The representative lettuce plants irrigated with PAW (left) and with tap water (right).

The fresh and dry weight represents the amount of biomass produced by the plant. The dry weight of above-ground parts of lettuce irrigated with PAW was higher compared to control. With increasing concentrations of H_2O_2 and NO_3^- in synthetic solution also the dry weight of above-ground part of lettuce increased (Fig. 3). The NO_3^- is main source of nitrogen for plant production of proteins and nucleic acids, therefore can be consider as a main PAW component responsible for weight increase. On the other side, H_2O_2 can take part in weight increase through the process of plant tissue lignifications.

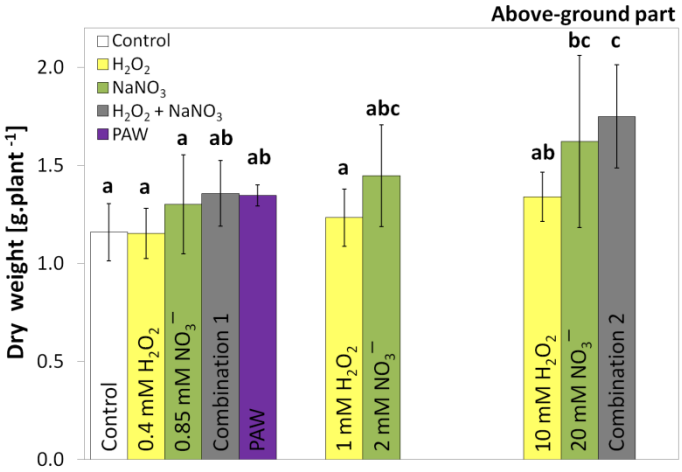

Fig. 3. Dry weight of above-ground part of lettuce irrigated with PAW, synthetic solutions of various concentration of H_2O_2 and/or NO_3^- and control irrigated with tap water.

Concentrations of photosynthetic pigments (chlorophylls and carotenoids) were higher in plants irrigated with PAW compared to control (Fig. 4). Chlorophylls are closely connected to photosynthesis rate, that PAW also positively affected (Fig. 5). The NO_3^- itself had stimulative effect on pigments, however H_2O_2 did not have. The NO_3^- as a source of nitrogen can contribute to the chlorophyll production in plants, unlike H_2O_2.

The activity of antioxidant enzymes reflects the level of oxidative stress that plant is facing. Here, we present the results of the SOD activity as one representative of the enzymes that are part of complex specialized system protecting plant cells against oxidation. SOD is intracellular antioxidant that decomposes $O_2^{\bullet-}$ to H_2O_2. The PAW can be a potential source of oxidation as it contains various reactive oxygen species and free radicals. However, we measured decrease activity of antioxidant enzymes in PAW irrigated plants compared to control (Fig. 6). It indicates that PAW did not increase the oxidative stress in plant cells. To compare the effect with the effect of synthetic solutions, the activity of antioxidant enzymes decreased with NO_3^- concentration and increased with H_2O_2 concentration in above-ground part and root of lettuce (Fig. 6). The H_2O_2 can contribute to elevated oxidative stress by its oxido-reduction potential. However, the high oxidative stress could be also the result of nutrient deficit; therefore NO_3^- could decrease the antioxidant enzymes activity through its nutrient function.

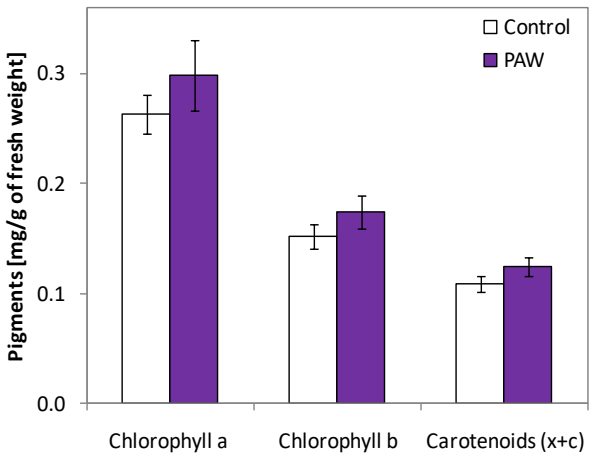

Fig. 4. Photosynthetic pigments content in above-ground parts of lettuce irrigated with PAW compared to control irrigated with tap water.

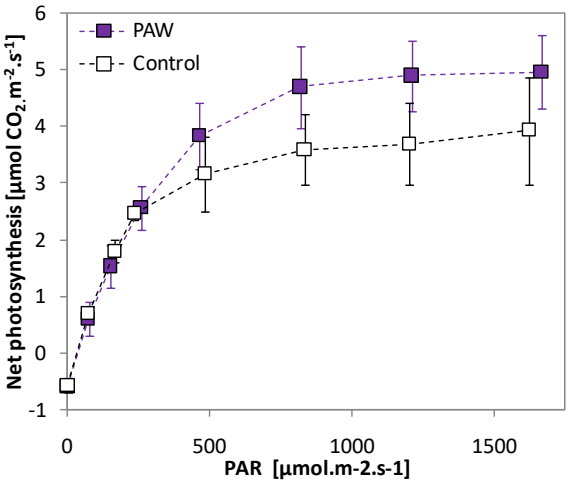

Fig. 5. Rate of photosynthesis in above-ground parts of lettuce irrigated with PAW compared to control irrigated with tap water.

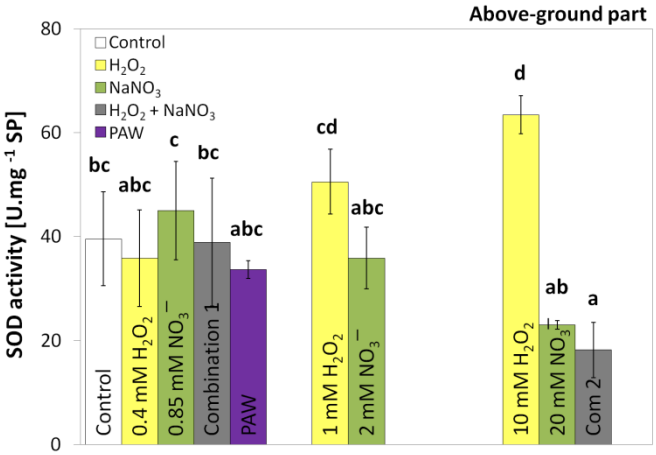

Fig. 6. Activity of superoxide dismutase (SOD) in the above-ground part of lettuce irrigated with PAW, synthetic solutions of various concentration of H_2O_2 and/or NO_3^- and control irrigated with tap water.

4. Conclusion

The effect of PAW generated by transient spark discharge on growth and important physiological and biochemical parameters of lettuce plants were investigated. The PAW effect was compared and correlated to the effect of H_2O_2 and/or NO_3^- synthetic solutions.

Lettuce irrigated with PAW in comparison to lettuce irrigated with chemically equivalent synthetic solution (Combination 1) had similar dry weight of above-ground part and root. However PAW induced higher photosynthetic pigment content, higher photosynthetic rate and lower activity of antioxidant enzymes.

The NO_3^- mainly contributed to the increase of dry weight, photosynthetic pigments content, rate of photosynthesis and overall better appearance of plants. The H_2O_2 induced increase of dry weight and antioxidant enzyme activity. Despite positive results obtained with the use of the PAW, the mechanism of its effect on plants is rather complex and need to be further studied.

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Effect of plasma activated water on lettuce

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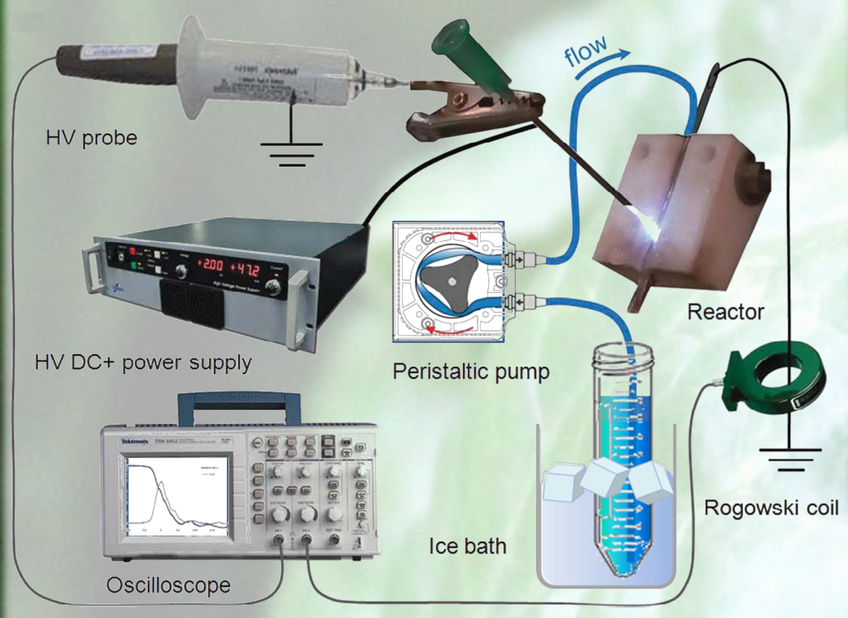
INTRODUCTION

Besides direct plasma treatment of seeds and plants the indirect treatment, i.e. effect of plasma activated water (PAW) has recently become of interest. The cold plasma generated by atmospheric pressure air discharges is a source of various reactive oxygen and nitrogen species (RONS), namely $^{\bullet}OH$, H_2O_2, NO, NO_2 that may dissolve in water while producing PAW. The long lived species in PAW (H_2O_2, NO_2^-, NO_3^-) may act as signal molecules in plant metabolism or be a source of nutrients. The effect of PAW generated by transient spark discharge on growth and important physiological and biochemical parameters of lettuce were investigated. The effect of PAW was compared with the effect of H_2O_2 and/or NO_3^- synthetic solutions of same and higher concentration as PAW.

EXPERIMENTAL SETUP



	H_2O_2 [mM]	NO_3^- [mM]	NO_2^- [mM]	pH
Control	-	~ 0.02	-	7.5
PAW	~ 0.42	~ 0.85	~ 0.38	7.5
H_2O_2	0.4	-		
	1	-		
	10	-		
NO_3^-	-	0.85		
	-	2		
	-	20		
mix I	0.4	0.85		
mix II	10	20		

Transient spark discharge
ambient air
activation time 1 min/mL
U_{max} = 10 - 13 kV
I_{max}= 6 - 8 A
f = 2 - 3 kHz
P ~ 6 W

Material
Tap water
Lettuce *Lactuca sativa* L.
var. capitata cv. Král máje I.

Cultivation
5 weeks in pots with soil
12-hour photoperiod
Temperature 24/18 °C (day/night)
Light intensity 120 µmol.m^{-2}.s^{-1}

PLANT ANALYSIS

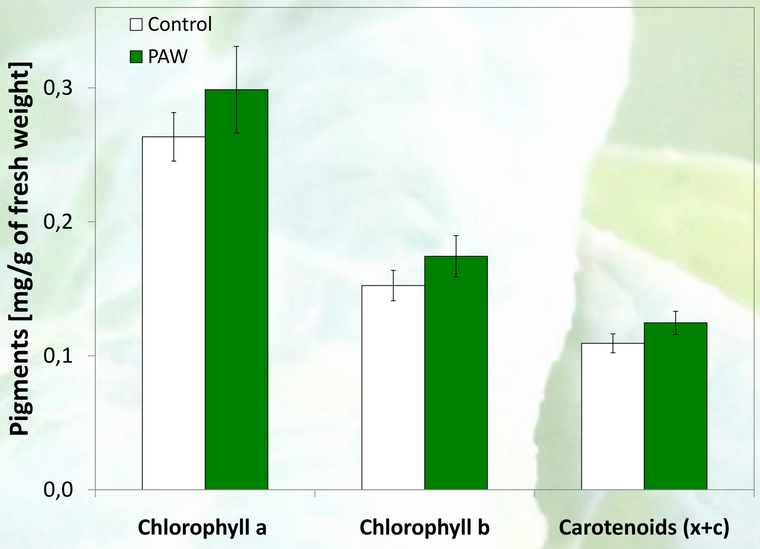
- Visual appearance - number and quality of leaves
- Fresh and dry weight of roots and above-ground parts of plants
- Photosynthetic pigment content in leaves
  chlorophylls (*a* + *b*), carotenoids (*x* + *c*)
- Photosynthesis rate (*CIRAS* - CO_2 - IR absorbance)
- Soluble protein content in roots and above-ground parts of plants
- Antioxidant enzyme activity (SOD, CAT, G-POX, APX)

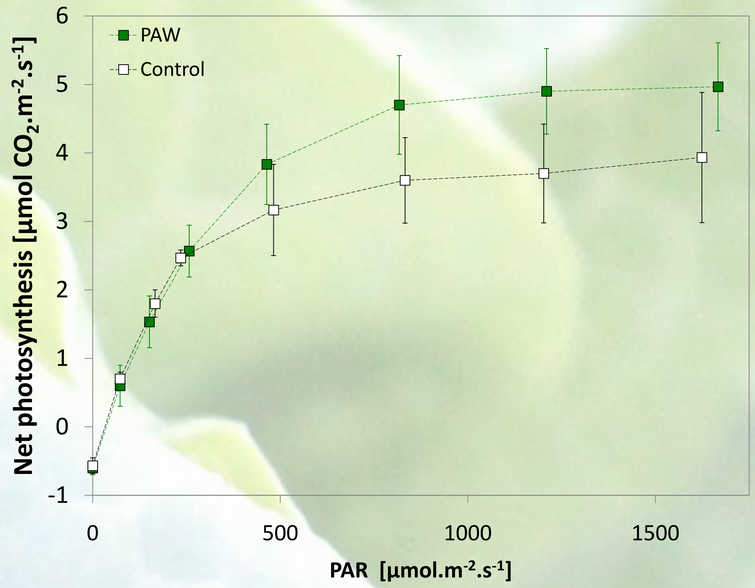
STATISTICS

- one-way ANOVA and multiple range test by LSD method
- lowercase letters indicate significant difference at p < 0.05

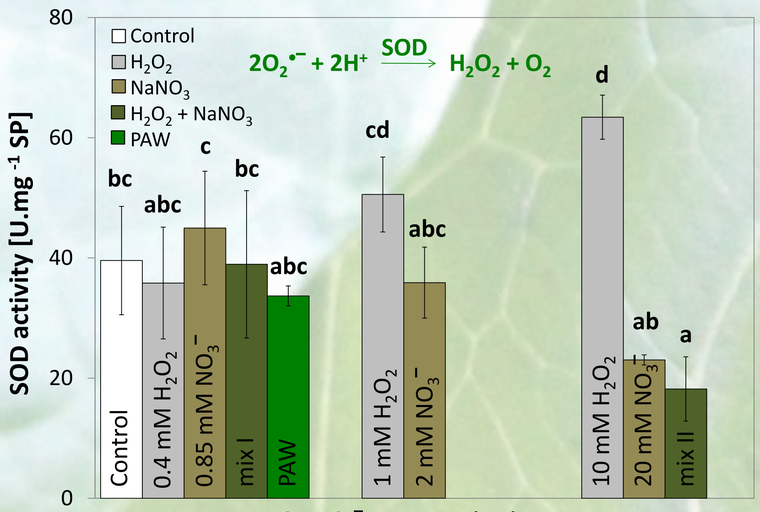
RESULTS

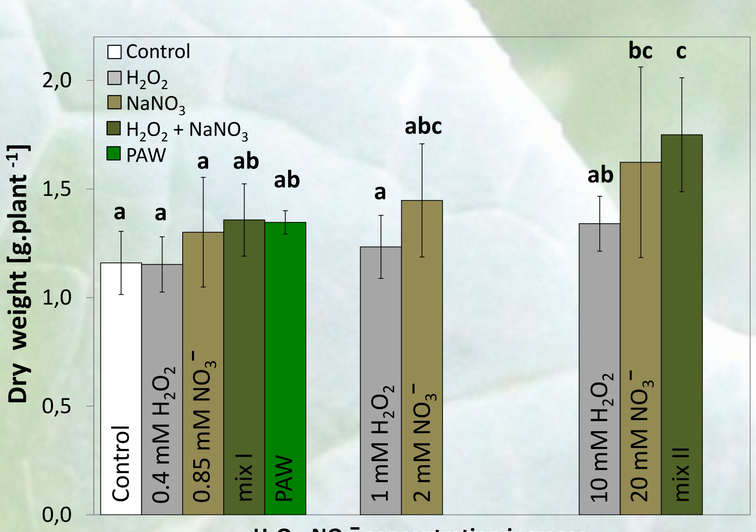
Lettuce irrigated with PAW had similar **number of leaves**, but differed in leaf size compared to control. Plants irrigated with synthetic solution containing NO_3^- had slightly higher number of green than senescent leaves compared to control. On contrary, high concentration of H_2O_2 negatively affected the appearance of plants. However, in combination with high NO_3^- concentration the positive effects of NO_3^- prevailed, as plants supplied with proper nutrition can better handle stress.





- Concentrations of **photosynthetic pigments** (chlorophylls and carotenoids) were higher in plants irrigated with PAW.
- Chlorophylls are closely connected to **photosynthesis rate**, that PAW also positively affected.
- The NO_3^- had stimulative effect on pigments, however H_2O_2 did not have.





- The **activity of antioxidant enzymes** in plants decreased with NO_3^- concentration and increased with H_2O_2 concentration, both in above-ground parts and roots.
- The H_2O_2 contributes to elevated oxidative stress by its oxido-reduction potential.
- High oxidative stress can be also the result of nutrient deficit. The NO_3^- through its nutrient function can decrease the antioxidant enzymes activity.

- **Dry weight** of above-ground part of lettuce increased with the increasing concentrations of H_2O_2 and NO_3^-.
- The NO_3^- is main source of nitrogen for production of proteins in plants, therefore can be considered as a main component in PAW responsible for weight increase.
- The H_2O_2 can take part in weight increase through the process of plant tissue lignifications.

CONCLUSION

- The **effect of PAW** and chemically equivalent H_2O_2 and/or NO_3^- **synthetic solutions** on growth and important physiological and biochemical parameters of **lettuce** were investigated.
- PAW had similar effect on **dry weight of above-ground parts and roots** of lettuce in comparison to H_2O_2 and NO_3^- synthetic solution (mix I).
- PAW induced higher **photosynthetic pigment content**, higher **photosynthetic rate** and lower **activity of antioxidant enzymes** copared to mix I solution and also to control.
- The **NO_3^-** mainly contributed to the increase of dry weight, photosynthetic pigments content, rate of photosynthesis and overall better appearance of plants, while **H_2O_2** induced increase of dry weight and antioxidant enzyme activity.

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