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Effect of plasma activated water on plant growth

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With increasing population and decreasing food sources there is demand for new approaches in agriculture. Low temperature plasma (LTP) is a promising technology able to enhance productivity and maintain quality of food and has a potential to be used in various agriculture applications. Beside direct plasma treatment of dry seeds for germination improvement [1] the plasma activated water (PAW) for stimulation of seed germination and plant growth has become recently popular, too [2]. LTP generated by electrical discharges in atmospheric pressure air is a source of various reactive species, free radicals and charged particles. When plasma is in the contact with water the reactive particles originated in the gas phase diffuse into the water and produce reactive oxygen and nitrogen species RONS ($^{\bullet}OH$, $O_2^{\bullet -}$, H_2O_2, NO_2^-, NO_3^-, $ONOO^-$/HONOO). The main species in the PAW responsible for the effect on seeds and plants are H_2O_2, NO_2^- and NO_3^-. They can act like signal molecules, are a potential source of nitrogen or may cause cross resistance against stress in plants [3].

The PAW was produced by a self pulsing transient spark discharge (TS) generated in a system with tap water repetitively flowing through the discharge zone [4]. We monitored the pH and concentration of H_2O_2, NO_2^-, NO_3^- in PAW by colorimetric methods via UV/Vis absorption spectrophotometry. The lettuce *Lactuca sativa* was used as a model plant. The plants were irrigated either with PAW or with H_2O_2 and/or $NaNO_3$ solutions to compare the individual and combined effect of the dominant species in PAW. After 5 weeks of cultivation in pots with soil in controlled conditions the growth parameters of the plants (number of leaves, fresh and dry weight), photosynthetic pigments (chlorophylls and carotenoids), rate of net photosynthesis, content of soluble proteins, and activity of antioxidant enzymes in leaves and roots of plants were analyzed.

The number of green leaves, dry weight of above-ground part and photosynthetic pigments content increased with increasing H_2O_2 and NO_3^- concentrations with more pronounced effect if both are present in the solution. Level of photosynthesis and protein content was found higher for the plants irrigated with PAW compared with the plants irrigated with a corresponding mixture of H_2O_2 and NO_3^-, while the results on dry weight and pigments were found comparable. Irrigation with PAW did not increase the antioxidant enzymes activities in above-ground part or roots of plants compared to the reference. The mechanism of RONS effect on plant metabolism and growth is complex and the obtained results need to be verified by further studies.

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References

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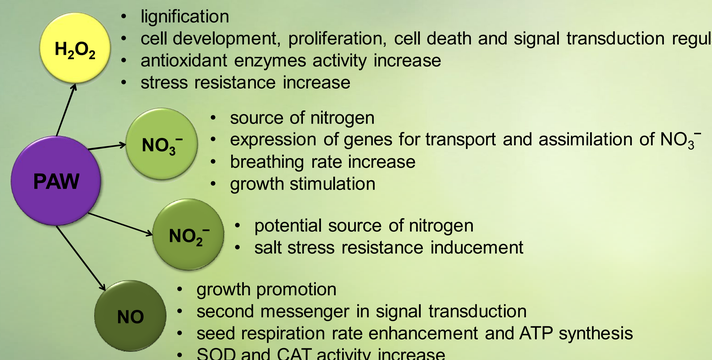
INTRODUCTION

Recently the cold plasma has found its use in various food and agriculture applications, e.g. treatment of seeds, plants and growing substrates including soil and water. Besides direct plasma treatment the effect of plasma activated water (PAW) for stimulation of seed germination and plant growth has become studied too. The PAW generated by the atmospheric pressure air discharges contains various types of reactive oxygen and nitrogen species (RONS), e.g. H_2O_2, NO_2^- and NO_3^-. These species may play an important role in seed and plant metabolism - serve as their essential nutrients or as signaling molecules controlling major physiological processes. The aim of this study was to investigate the effect of PAW generated by the transient spark discharge on growth and some important physiological and biochemical parameters of lettuce plants and compare it to the effect of H_2O_2 and/or $NaNO_3$ solutions.

The role of RONS in plant metabolism [1, 2]:

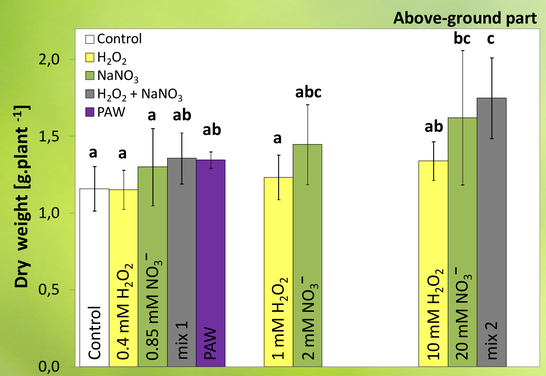


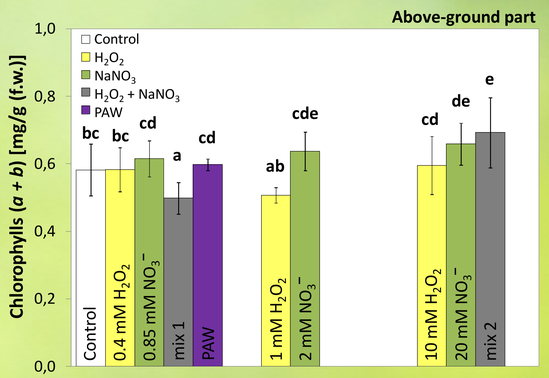
- lignification
- cell development, proliferation, cell death and signal transduction regulation
- antioxidant enzymes activity increase
- stress resistance increase

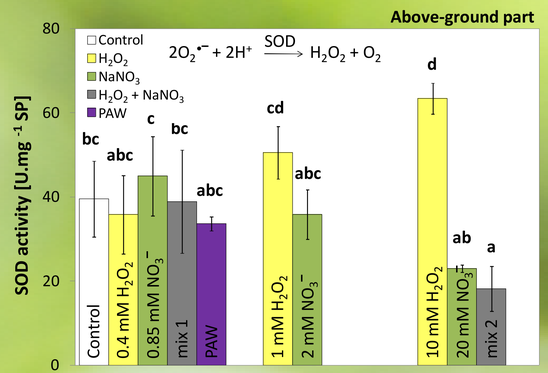
- source of nitrogen
- expression of genes for transport and assimilation of NO_3^-
- breathing rate increase
- growth stimulation

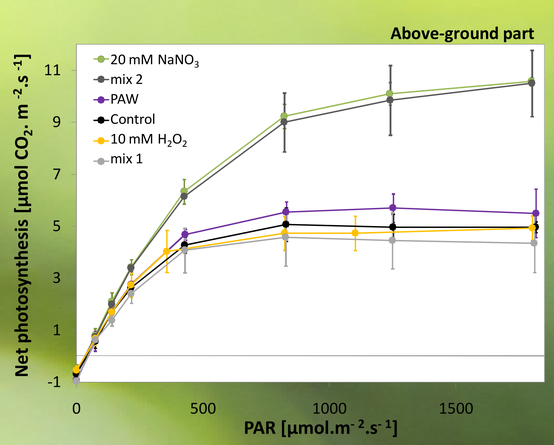
- potential source of nitrogen
- salt stress resistance inducement

- growth promotion
- second messenger in signal transduction
- seed respiration rate enhancement and ATP synthesis
- SOD and CAT activity increase

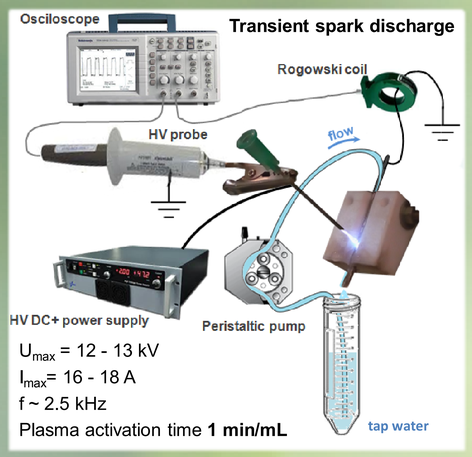








MATERIAL & METHODS



U_{max} = 12 - 13 kV
I_{max}= 16 - 18 A
f ~ 2.5 kHz
Plasma activation time **1 min/mL**

- Control – tap water
- **Plasma activated water (PAW) 1 min/mL**
- **H_2O_2** (0.4, 1, 10 mM)
- **$NaNO_3$** (0.85, 2, 20 mM)
- **mix 1** = 0.4 mM H_2O_2 + 0.85 mM $NaNO_3$ (~ 1 min/mL)
- **mix 2** = 10 mM H_2O_2 + 20 mM $NaNO_3$ (~ 28 min/mL)

Watering ~ 10 - 30mL

Lettuce
Lactuca sativa L.

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

Cultivation

PLANT ANALYSIS

- Visual - 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*)
- Rate of photosynthesis (*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

Data were analyzed by one-way ANOVA and multiple range test by LSD method. The groups marked with different lowercase letters indicate significant difference at probability level of $p < 0.05$.

RESULTS

- Plants in all variants had an average of 23 **leaves**, but differed in leaf size. Variants irrigated with NO_3^- solution had slightly more green than senescent leaves compared to the control.
- **Dry weight** of above-ground part of plants increased with increasing both H_2O_2 and NO_3^- concentration, with more pronounced effect if both were present in the solution (mix or PAW). Similar trend, although not so significant, was observed in dry weight of roots. H_2O_2 takes role in the plant cell wall lignification, that may contribute to the dry weight increase.
- The presence of NO_3^- in the solution had slightly stimulating effect on **photosynthetic pigments** (chlorophylls and carotenoids) in the leaves. On the other hand, the H_2O_2 solution alone did not stimulate the photosynthetic pigments production.
- The PAW irrigated lettuce showed higher **photosynthesis rate** compared to the chemical equivalent of PAW and the control. The stronger effect of NO_3^- compared to H_2O_2 on photosynthetic rate was due to the higher pigment content.
- **Soluble protein** content was higher in above-ground parts than root part of the plants and it was more affected by NO_3^- concentration with additive effect of H_2O_2 in comparison to the control.
- **Activity of antioxidant enzymes** decreased with the increasing NO_3^- concentration and decreasing H_2O_2 concentration in both root and above-ground part of plants. On the other hand the PAW did not increase the antioxidant enzymes activities in above-ground part or roots compared to the control. These results confirm that irrigation with PAW did not increase the oxidative stress in lettuce. Higher oxidative stress in the control could be the result of nutrient deficit, as the tap water contain only minimum of NO_3^-.
- **NO_3^-** mainly contributed to the higher dry weight, chlorophyll content, photosynthesis rate and overall better appearance of plants as it is the main source of nitrogen for proteins, chlorophylls and nucleic acids production.
- **H_2O_2** took part in weight increase, and increased antioxidant enzyme activity, however did not induce any change in pigments.

CONCLUSION

The effect of PAW generated by transient spark discharge on lettuce was investigated and compared with the effects of chemical equivalents of H_2O_2 and/or $NaNO_3$ solutions. Lettuce irrigated with PAW had similar dry weight of root and above-ground part compared to its chemical equivalent. However PAW induced higher content of photosynthetic pigments, higher photosynthetic rate and lower activity of antioxidant enzymes. The mechanism of RONS effect on plant metabolism and growth is relatively complex and the obtained results need to be verified in further studies. Moreover the effect of PAW could be more pronounced in hydroponic cultivation or foliar irrigation, where the plant are in direct contact with RONS in PAW.

References

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