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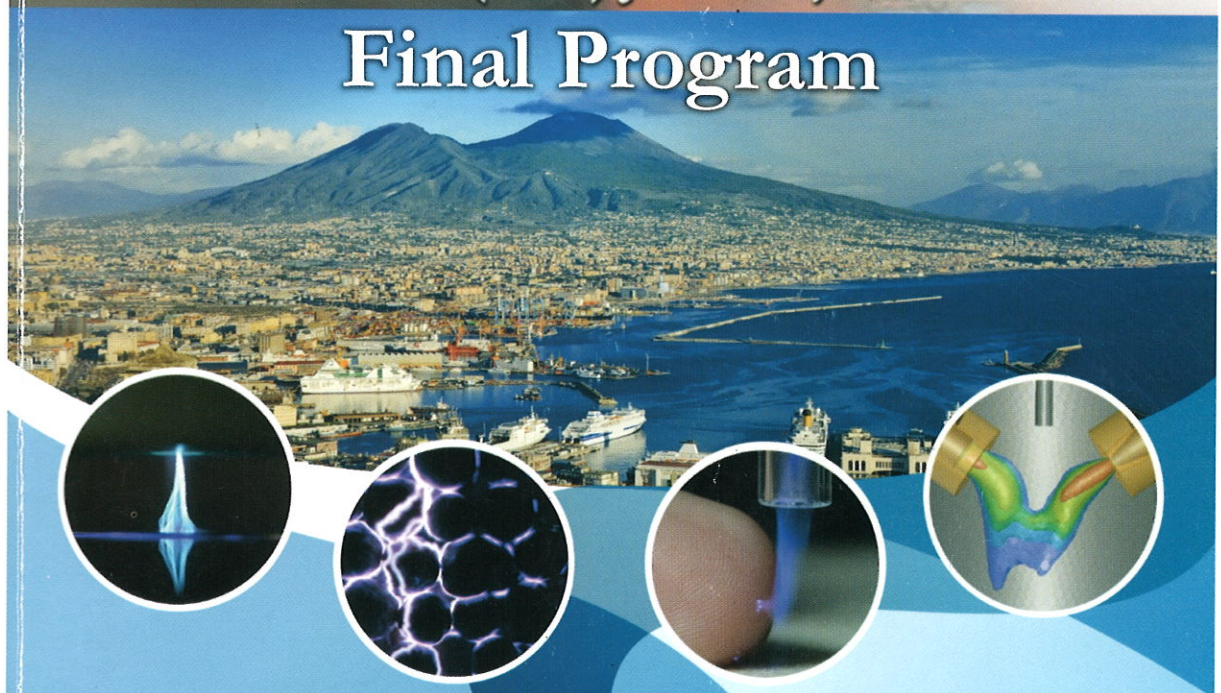

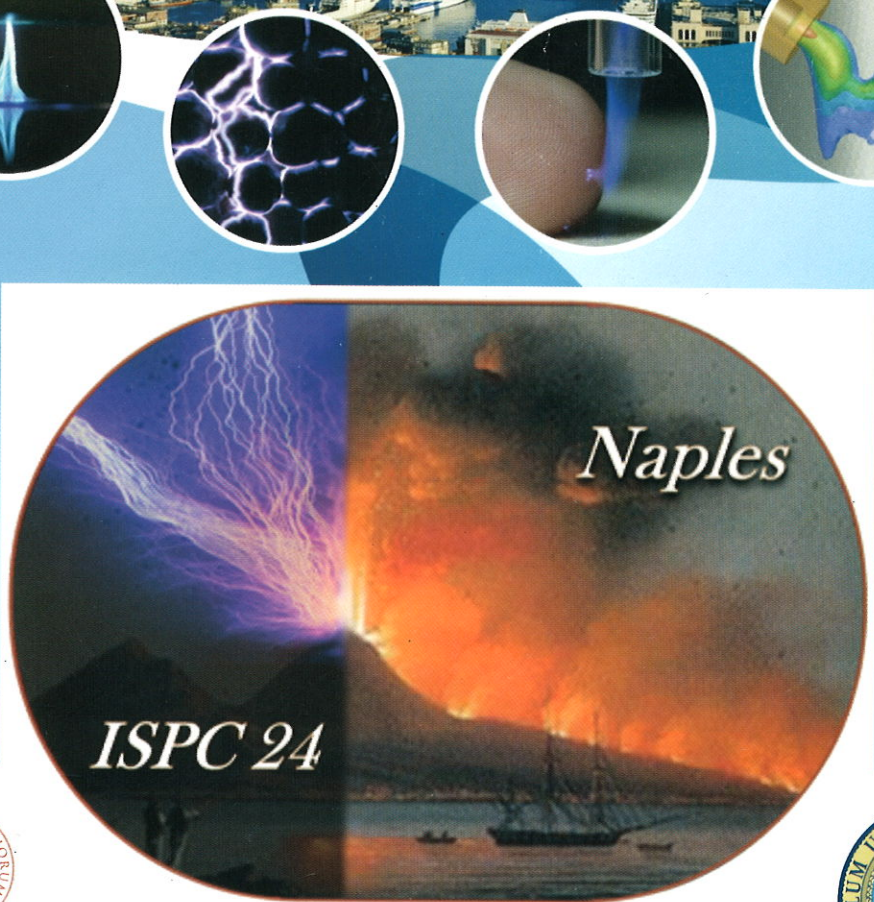

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Effects of plasma activated water on wheat and lettuce: Germination, growth parameters, photosynthetic pigments, soluble protein content and antioxidant enzymes activity

K. Hensel[1], K. Kučerová[1], M. Saganová[2], Ľ. Slováková[2] and M. Henselová[2]

[1]*Faculty of Mathematics, Physics and Informatics, Comenius University,*
[2]*Faculty of Natural Sciences, Comenius University*
Mlynská Dolina, 842 48 Bratislava, Slovakia

Abstract: The effect of plasma activated water (PAW) generated by transient spark discharge on wheat and lettuce was investigated *in vitro* and *in vivo*. Water uptake, germination of seeds, growth parameters of seedlings, as well as the content of soluble proteins and photosynthetic pigments, photosynthesis rate, and activity of antioxidant enzymes were analyzed. The effect of PAW was compared to the effects of chemically equivalent synthetic solutions containing the main reactive species (H_2O_2, NO_3^-).

Keywords: wheat, lettuce, plasma activated water, germination, chlorophylls, photosynthesis, soluble protein, antioxidant enzymes

1. Introduction

Cold plasma has shown promising potential in various agricultural applications [1, 2]. The application of the CP is either direct (seeds or plants are in a direct contact with plasma) or indirect (seeds or plants are exposed to plasma treated/activated gas or water). The plasma in direct contact with the seed can stimulate its germination and growth and subsequent seedlings and fruit yield, change enzymatic activity in developing seeds, change secondary metabolites content and also reduce phytopathogenic microflora from the seed surface. The indirect plasma treatment/activation of seeds and plants by the CP is far less studied than the direct plasma effect. The PAW can be generated by electric discharges directly in water, but more often in gas in a contact with water, usually above the water surface. The CP produces various gaseous and aqueous reactive oxygen and nitrogen species (RONS), that affect and control numerous processes in seeds and plants, including their germination, growth, development, and response to a stress. Among these RONS, hydrogen peroxide H_2O_2 and nitrates NO_3^- are mainly considered being the species responsible for the enhancement of seed germination and growth of plants, and the activity of the PAW is usually determined by their concentration. The quality/activity of the PAW can be affected in general by various parameters, such as discharge type and power, composition of gas mixture and gas flow rate, and type of water.

The objective of the study was to investigate the effect of the PAW generated by a transient spark discharge in ambient air in contact with water on germination and growth of wheat (*Triticum aestivum* L.) and lettuce (*Lactuca sativa* L). We analyzed water uptake by seeds, germination of seeds, and growth parameters of seedlings and plants (wheat) grown in different cultivation conditions *in vitro* and *in vivo*. The effect of the PAW on seeds and seedlings was investigated along with the monitoring of RONS concentration in the PAW during cultivation. From the physiological parameters we determined the content of soluble proteins and photosynthetic pigments, photosynthesis rate and monitored the antioxidant enzymes activity (in both wheat and lettuce). We compared the effect of the PAW with the effect of H_2O_2 and/or NO_3^- chemically equivalent synthetic solutions to understand the individual role of reactive species in the PAW (lettuce).

2. Experimental setup and methods

The PAW was generated by positive DC driven self-pulsing transient spark (TS) discharge at atmospheric pressure in air. The TS was generated in a reactor with water circulating through the discharge zone and driven by the pump. To compare the results obtained for different activation times (min) and water volumes (mL) we use the term *water activation time* expressed in units of min/mL. Chemical composition of PAW was measured by colorimetric methods using UV/Vis absorption – H_2O_2 by titanium sulfate, and NO_2^- and NO_3^- by Griess reagents. Water uptake by seeds was determined after the imbibition as the increase of seed weight. Cultivation *in vitro* was performed in Petri dishes, with seeds imbibed in PAW for 3h and then placed on filter paper regularly moistened with freshly prepared PAW and cultivated for 6 days. At the end of cultivation the germination, fresh and dry weight of seedlings, length of roots and shoots was measured and vigor indices were evaluated. Germination percentage was evaluated as a ratio between number of germinated seeds and total number of seeds. Randomly selected seedlings were weighted to determine the fresh weight, and after drying also the dry weight. Cultivation *in vivo* was performed in pots filled with a perlite for 4 weeks in controlled conditions in growth chamber and irrigated with the PAW of different activity

(0.5, 1 and 2 min/mL). The number and quality of leaves was evaluated and leaf area was calculated. Fresh and dry weights were determined for root and above-ground part of the plants in the same manner as for *in vitro* experiments. Photosynthetic pigments (chlorophylls and carotenoids) in the leaves were evaluated based on the absorbance measured by UV/Vis spectrophotometer. The photosynthesis rate was performed by infrared analyzator based on rate of CO_2 exchange in dependence on light intensity. Total soluble protein content was measured using Coomassie Brilliant Blue dye, and the activity of antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT), and guaiacol peroxidase (G-POX) was measured according to standardized assays using UV/Vis spectrophotometer. More details on the plasma reactor, the used material, chemical and biological analysis can be found in [3].

3. Experimental results and discussion

The concentration of the RONS determines the activity of the PAW and the effects induced in seeds and plants. The concentrations increased with the increasing water activation time and decreases once the plasma discharge is stopped. Typical concentrations of RONS in the PAW after water activation time of 1 min/mL were as follows: H_2O_2 ~ 0.42 mM, NO_2^- ~ 0.38 mM, and NO_3^- ~ 0.85 mM.

The water uptake by seeds (wheat) was monitored for 24h based on the changes in the weight of seeds. The most intense water uptake was observed during the first 3h, while later after 6h and 24 h it was significantly smaller. The difference between uptake of the deionized and tap water was observed and attributed to differences in their osmotic potentials. Faster hydration of seeds can cause quicker changes in enzymatic state of the germinating seeds. The maximum stimulation effect of the PAW on germination (wheat) by 103% was observed after 24h for 0.5 min/mL PAW, then toward the 6^{th} day the effect was slowly attenuated. The stimulating effect of the PAW on germination was probably due to the preferential intake of H_2O_2 from the PAW during seed imbibition and subsequent cultivation. H_2O_2 may stimulate respiration and metabolic activities, help water diffusion or oxidize germination inhibitors. The positive effect of the PAW can be also due to other reactive species, such as O, OH, NO_2^- and NO_3^-. Faster water uptake together with faster germination usually leads to pronounced growth of seedlings. We found the most pronounced effect with 0.5–1 min/mL PAW, where average length of seedlings increased by 6–7% and fresh weight by 19%.

In vivo cultivation was performed in nutrient free substrate for prolonged period of 4 weeks. No obvious differences between control and plants irrigated with the PAW was found, however subsequent detailed analysis showed several differences in their growth parameters (leaf area and number of green and senescent leaves), photosynthetic pigments, soluble photosynthetic pigments, soluble protein content, and antioxidant enzymes activity. Plants (wheat) irrigated with 0.5 and 2 min/mL PAW had higher leaf area by 22 and 23%, respectively, and greener and less senescent leaves when compared to control. Plants (lettuce) irrigated with synthetic solution of NO_3^- had also higher number of green leaves compared to control, while high concentration of H_2O_2 negatively affected the appearance of plants. However, in combined solution with high NO_3^- concentration, the positive effects of NO_3^- prevailed. The premature physiological aging of leaves could be caused not only by lack of nitrogen but also by deficiency of other macro elements needed for intense growth. No significant difference in dry weight was found (wheat), however stimulating effect was observed in the increase of above-ground part of the plant and less in its root (both wheat and lettuce). This finding shows that nitrogen absorbed by plants is efficiently used especially for active photosynthesis and assimilation in favor of biomass production.

The content of photosynthetic pigments (chlorophylls and carotenoids) also increased with the PAW activity. Plants irrigated with 2 min/mL PAW showed 17 and 12% increase of chlorophylls (a+b) and carotenoids (x+c), respectively. The increase is a sign of increased rate of photosynthesis and total plant metabolism. We assume that the increase of carotenoids in plants irrigated with the PAW is not a sign of elevated oxidative stress, but rather the result of higher concentration of the RONS. The analysis of the rate of photosynthesis in above-ground parts showed the positive affected of irrigation with PAW (lettuce). In addition, the experiments with synthetics solutions (lettuce) showed the stimulative effect on pigments comes mainly from NO_3^-, while H_2O_2 showed no effect.

Soluble proteins play an important role in the growth of the plants and are very important component of numerous plant enzymes that reflect the overall plant metabolism. We observed significant increase ~ 43% and ~19% in total protein proteins content in root and in above ground part of plants irrigated with 0.5 min/mL PAW, respectively. The increase of proteins is probably mainly caused by NO_3^-, although other RONS may also affect the protein concentration.

The activity of antioxidant enzymes was done to find whether the PAW can cause oxidative stress to plants. The increase in oxidative stress usually induces increased expression of antioxidant enzymes. The highest activity of enzymes was found in control, as the result of long lasting deficit of nutrients. The activity of the enzymes decreased with the increasing PAW activity, that is, with the increasing concentration of the RONS. Significant reduction of SOD activity to 92%, CAT activity to 75% and G-POX activity to 76% in above-ground was found for 0.5 min/mL PAW, respectively. With respect also to

other reported physiological parameters we assume that our PAW did not induce the oxidative stress to the plants under conditions in which they were grown. In addition, the experiments with the synthetic solutions showed the activity of antioxidant enzymes in above-ground part and root (lettuce) decreased with NO_3^- concentration and increased with H_2O_2 concentration.

4. Conclusion

Atmospheric pressure cold plasma represents a promising method in agriculture. We investigated the effect of plasma activated water (PAW) generated by the transient spark on seeds and plants of wheat and lettuce. Water uptake, germination, various growth parameters, and vigor indices, as well as photosynthetic pigments, photosynthesis rate, content of soluble proteins and activity of antioxidant enzymes were studied and evaluated with respect to the type of the PAW and its activity both *in vitro* and *in vivo* conditions. The effect of PAW was briefly compared with the effect of H_2O_2 and/or NO_3^- synthetic solutions. The maximum improvement of seed germination, fresh, and dry weight and length of seedlings analyzed *in vitro* were observed for ~ 0.5 min/mL water activation time. The improvement in response to the use of the PAW is probably due to the increase in water uptake that induces faster seed's nutrition reserve utilization and metabolization of nitrogen species during vegetative growth. The effect of the PAW on seeds was correlated with the PAW activity and its chemical composition, i.e. concentrations of the RONS (H_2O_2, NO_2^-, and NO_3^-). The seeds cultivated in the PAW interact with H_2O_2 mainly in the early growth stages during imbibition and germination, while NO_2^- and NO_3^- are metabolized once the seeds start to germinate. The plants irrigated with the PAW and cultivated for 4 weeks in nutrient free substrate *in vivo* usually showed more green leaves, higher chlorophyll and carotenoids content and lower activity of antioxidant enzymes. The comparison of results obtained with PAW and chemically equivalent synthetic solutions showed NO_3^- mainly contributed to the increase of dry weight, photosynthetic pigments content, photosynthesis rate and overall better appearance of plants, while H_2O_2 induced increase of dry weight and antioxidant enzyme activity. The results demonstrate that RONS in the PAW serves not only as nutrients but they may also act like signaling molecule and have the potential to enhance the germination of seeds and subsequent growth of the seedlings. For prospective application of the PAW for irrigation of seeds and plants further optimization of water activity with respect to the specific plant growing conditions is necessary.

5. Acknowledgement

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