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PLASMA ACTIVATED WATER GENERATED BY TRANSIENT SPARK AIR DISCHARGE: CHEMICAL PROPERTIES AND APPLICATION IN SEED GERMINATION AND PLANT GROWTH

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Abstract: Cold plasmas generated by electrical discharges in air at ambient temperature and atmospheric pressure generate reactive oxygen and nitrogen species (RONS) that in contact with water generate the plasma activated water (PAW). PAW produced by pulsed discharge electrospray was chemically analysed and its effects on maize seed germination and growth were investigated. Higher RONS concentrations in PAW resulted in enhanced germination and plant growth.

Cold plasma, electrospray, plasma activated water, reactive oxygen and nitrogen species.

1. Introduction

Cold plasma generated by electrical discharges in air at atmospheric pressure may cause strong oxidation and antimicrobial effects and induce other beneficial effects in food processing and agriculture [1-3]. This plasma is an efficient source of radicals and reactive species often coupled with the effects of electric fields and radiation, and opens up many new applications, especially in disinfection and sterilization, juice pasteurization, stimulation of seed germination and plant growth [4, 5]. It has shown immense potential as a simple, safe, and environmental friendly alternative to various chemical processes used in the food processing and agriculture [6]. These properties have led researchers to study the action of this plasma on aqueous solutions in general and water in particular. Its action on water has given very positive results with the in situ generation of highly reactive species in contact at the gas-water interface, thus generating the reactive oxygen and nitrogen species in this water [1]. In this study we evaluated the effect of two water flow rates and electrospray method through two needles on the chemical composition of plasma activated water generated by transient spark discharge and investigated the effect of PAW produced on the germination and growth of maize. Positive effects of similar PAW prepared by transient spark air discharge in water circulation system have been reported on wheat seeds [7].

2. Materials and methods

a- Plasma device and PAW preparation procedure

i- The experimental setup for DC-driven transient spark discharge in point-to-plane geometry is shown in Fig. 1. The discharge was generated in ambient air through the water-spray system configuration. Two high voltage (HV) hypodermic hollow needle electrodes enabled us to inject water through the discharge zone with constant flow rates (0.5 and 1.0 $mL.min^{-1}$), and the water was electro-sprayed through the active zone of the discharge and then collected under the metallic mesh. The inter-electrode spacing from the HV needles to the grounded mesh electrode was usually kept around 8 mm. The discharge voltage was measured by a HV probe Tektronix P6015A. The discharge current was measured by a Rogowski current monitor Pearson Electronics 2877. The current and voltage signals were processed by a digitizing 200 MHz oscilloscope Tektronix TDS 2024.

ii- Maize (*Zea Mays L. var Saccharata*) seeds were used to investigate the PAW effect on germination and plant growth (in vitro and in vivo), using two flow rates 0.5 and 1.0 $mL.min^{-1}$ (PAW 0.5 and PAW1.0 respectively) and the effect of long-lived reactive oxygen and nitrogen species (RONS) concentrations was evaluated.

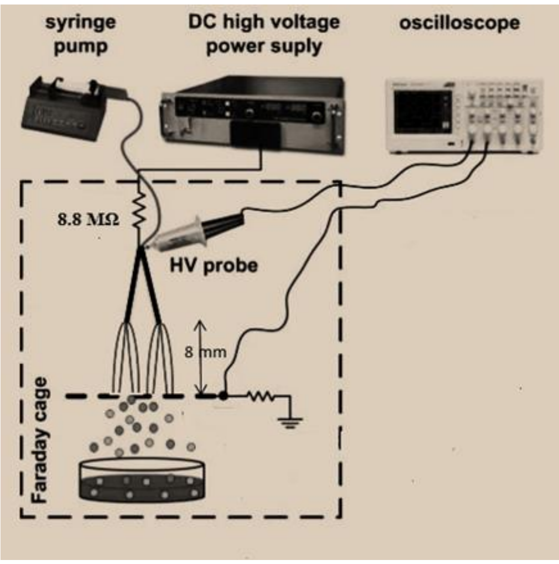

Fig. 1. The experimental setup with electro-spray of water through two needles and two parallel transient spark discharges

b- Performed water analysis

The detection of reactive species (hydrogen peroxide, nitrites/nitrates) was performed by UV/VIS absorption spectroscopy colorimetric methods (Shimadzu UV-1800 Spectrophotometer) as follows:
i- Determination of hydrogen peroxide (H_2O_2): H_2O_2 reacts in the presence of sodium azide (NaN_3 to remove interacting nitrites) with a reagent titanyl oxysulphate ($TiOSO_4$) and the resulting yellow product with a maximum absorption (407 nm), is directly proportional to the concentration of H_2O_2.
ii- Determination of nitrite and nitrate (NO_2^- and NO_3^-):
Nitrite reacts with Griess reagents and produces a peak of azo purple product at an absorption maximum (540 nm), which is proportional to the concentration of NO_2^-. Nitrates are converted to nitrites by nitrate reductase and measured as above.
iii-The change of pH in plasma treated water was measured by pH probe (WTW 3110)

c- Seed procedure
i- *In vitro* tests
Seeds of maize (*Zea Mays L. var Saccharata*) were soaked during 6 h in tap water or PAWs. After that 10 seeds were placed in petri dishes (90 mm diameter) containing two layers of filter paper and moisturized with 5mL of tap water or PAWs. Germination was performed at 24 ± 2 °C in darkness for 3 days. All experiments were made with 4 replicates and during germination tap water or PAWs were added to each petri dish every other day to maintain constant moisture.
ii- *In vivo* tests
Seeds were sown in experimental pots (100 ml) containing soil. Each pot was watered with tap water or PAWs to keep the constant moisture level of soil. The plants were cultivated for 7, 10 and 14 days in controlled growth conditions: 23 ± 2 °C, a 12 h light/12 h dark.

d- Statistical Analysis

Differences among different treatments were evaluated using the one-way analysis of variance (ANOVA). Results were significant at $*P < 0.05$; $**P < 0.01$. Values are expressed as a mean ± standard deviation (SD).

3. Results and Discussion

a- Transient spark discharge parameters

The typical voltage and current waveforms of the positive TS discharge in 8 mm gap with electrospray of water are shown in Fig. 2. The typical pulse repetition frequency ~1 kHz and pulse duration ~25 ns. Transient spark was operated with water electrospray flow rates 0.5 and 1 mL/min.

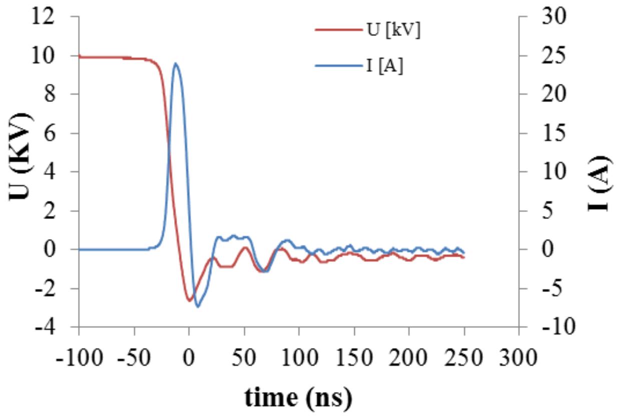

Fig. 2. The typical voltage and current pulse waveforms of the transient spark with water electrospray

b- RONS concentrations and pH in water and PAW

As shown in Fig. 3a, reactive oxygen and nitrogen species were detected in the control with the concentrations of 0.0, 0.0 and 235.6 µM respectively for hydrogen peroxide (H_2O_2), nitrite (NO_2^-) and nitrate (NO_3^-). The chemical analysis showed an increase of RONS concentrations in the plasma activated water. The concentrations of treated water are 449.6, 1075.1, 1578.9 µM, respectively, for H_2O_2, NO_2^- and NO_3^- for PAW 0.5 and 317.2, 575.1, 848.2 µM, respectively, for hydrogen peroxide, nitrite and nitrate for PAW 1.0. Fig. 3b shows a slight difference of pH between the control and plasma activated waters.

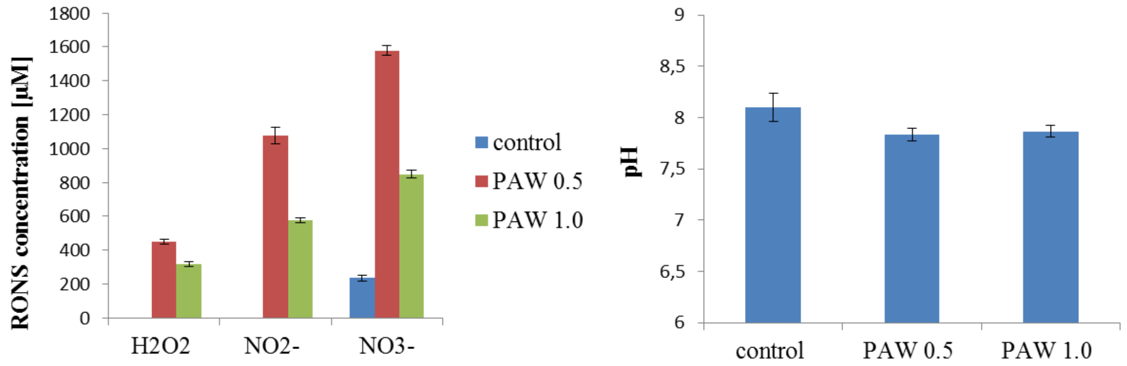

Fig. 3. a)- Hydrogen peroxide (H_2O_2), nitrite (NO_2^-), and nitrate (NO_3^-) concentrations in the PAW b)- pH measured in water before and after plasma treatment.

c- "*In vitro*" seed germination test (petri dishes)

Fig. 4a shows the results of the germination rate of maize after one day, in the figure the germination rate was 87.5, 100% and 95.8 for the Control, PAW 0.5, and PAW 1.0, respectively, and there was significant a difference between the control and PAW 0.5. The improvement effect of PAW was 14.28 and 9.52 %, respectively, for PAW 0.5 and PAW 1.0. Figs. 4b and 4c show the root and shoot length after 6 days of seed cultivation with PAW. The maximum enhancement 1.09 times and 1.37 times, respectively, for the root and shoot length is obtained with PAW 0.5. The fresh weight of seedling after 6 days was 302.7 and 321.3 mg for the control and PAW 0.5, respectively, as shown in Fig .4d.

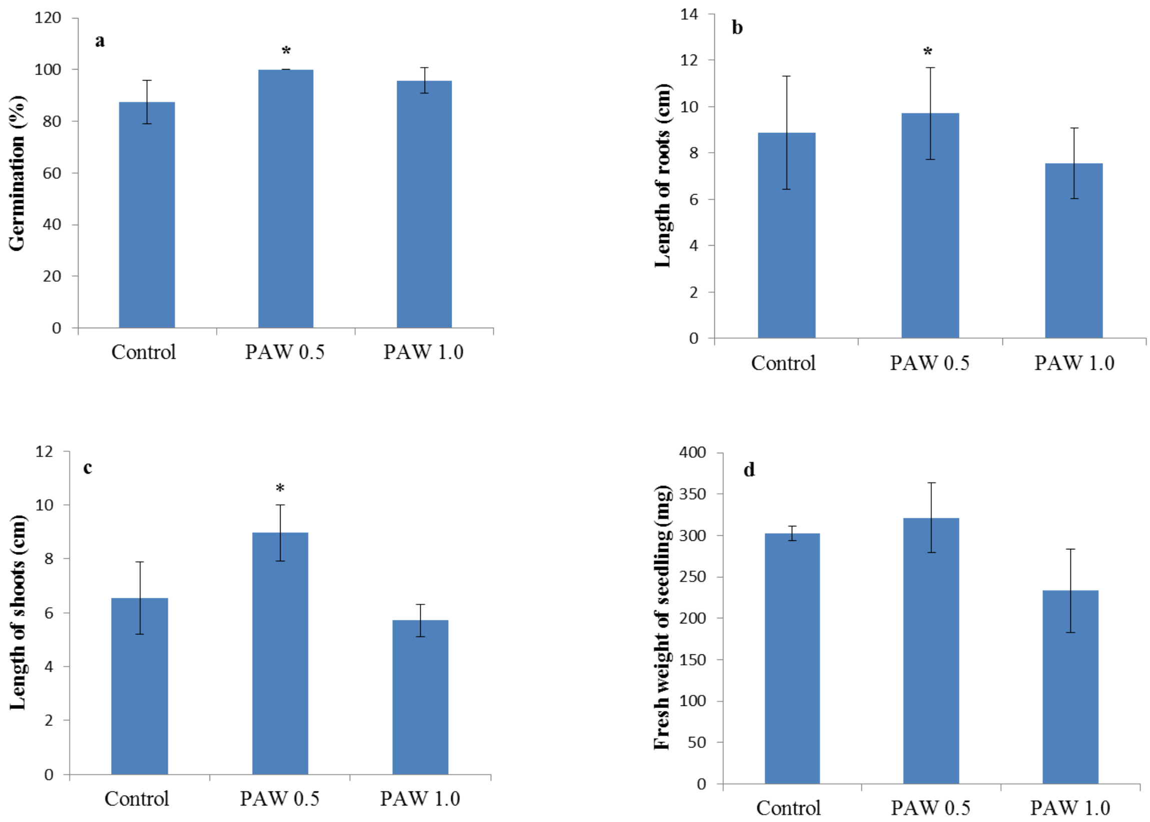

Fig. 4. a) Germination rate of maize after 1 day, b, c and d) root length, shoot length and fresh weight of seedling after 6 days of seed cultivation.

d- "*In vivo*" plant growth (sowed in the soil)

As can be seen in Fig. 5, the best plant growth enhancement results were obtained for the plants watered with PAW compared to control. The higher results have been reached with PAW 0.5 where the concentration of RONS was higher than in PAW 1.0. For instance the plants length for PAW 0.5 was 2.1, 1.8 and 1.3 times higher, respectively, on day 7th, 10th and 14th as compared to control.

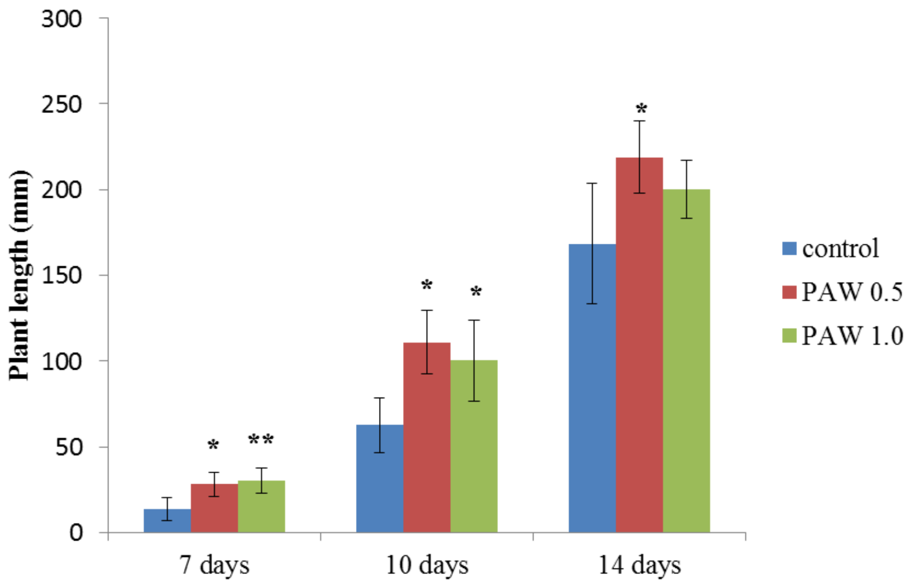

Fig. 5. Plant length of maize after 7-14 days of sowing in soil

4. Conclusion

The plasma activation of water treated by the transient spark discharge at ambient air and atmospheric pressure induces chemical changes in the water mostly dominated by production of hydrogen peroxide, nitrate and nitrate. We investigated the effects of PAW on maize seed germination and plant growth. The following conclusions are obtained:

The concentration of RONS depends of water flow rate during the plasma treatment.

PAW induces higher germination rate and enhances the plant length; the stronger effect was obtained for PAW 0.5 with higher RONS concentrations.

The long lifetime species in PAW are probably responsible for the growth enhancement.

5. Acknowledgements

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