Plasma activated water and airborne ultrasound treatments for enhanced germination and growth of soybean

C. Lo Porto,[1] D. Ziuzina,[2] Agata Los,[2] Daniela Boehm,[2] Fabio Palumbo,[3] Pietro Favia,[3,4] Brijesh Tiwari,[5] Paula Bourke,[2] Patrick J. Cullen,[2,6]

1. Department of Chemistry, University of Bari "Aldo Moro", Via Orabona 4, 70126 Bari, Italy;
2. School of Food Science and Environmental Health, Dublin Institute of Technology, Dublin, Ireland;
3. Institute of Nanotechnology, National Research Council of Italy, c/o Department of Chemistry, University of Bari "Aldo Moro", Via Orabona 4, 70126 Bari, Italy;
4. Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari "Aldo Moro", Via Orabona 4, 70126 Bari, Italy;
5. Food Chemistry and Technology, Teagasc Food Research Centre, Dublin, Ireland;
6. Department of Chemical and Environmental Engineering, University of Nottingham, United Kingdom.

Abstract: The effect of two novel technologies, also in combination, on germination and growth of soybeans has been investigated. On one side, ultrasound treatment of the seeds increased water uptake without altering the morphology and the wettability of the seed coat, but also induced slight chemical modifications of the outer part of the seed. Plasma-Activated Water (PAW), obtained from treating water with non-thermal atmospheric-pressure plasma in air, increased the rate of germination and subsequent plant growth.
Keywords: Plasma-Activated Water (PAW), Ultrasound, Soybeans, Seed germination, Plant growth.

1. Introduction

Soybean (*Glycine max*) is a legume whose worldwide production is increasing due to its multiple uses, therefore, the need to improve the productivity of this cultivation is urgent.

Non-thermal atmospheric-pressure plasma is becoming increasingly important in agriculture. [1-2] Between its numerous applications in this field one of the most promising is the production of Plasma Activated Water (PAW) for irrigation. Plasma in water produces Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) and lowers the pH, thus acting as an antibacterial agent and as a fertilizer. [3-5] A high oxidation reduction potential (ORP) combined with a low pH is found to be responsible for the antibacterial activity of PAW. The fertilizing properties depend on the presence of two important species: H_2O_2 and NO_3^-. Compared to other fertilizers, PAW can be manufactured cheaply from water and air, which along with the absence of organic compounds and pollutants makes the technique attractive.

In recent years ultrasound has gained greater attention as a technology to stimulate plant germination with many examples reported in literature on seeds (of different plant species) exposed to high energy ultrasound using a sonication water bath. [6-11] Airborne sonication, on the other hand, is less studied and requires a very powerful and efficient power source to compensate for the greater power loss occurring during the air propagation of the waves. This technique allows the treatment of seed by modification of their coat without damage to the seed, with the dry nature of the process permitting post treatment storage.

The aim of this work was to study combined effect of PAW and airborne sonication on water absorption, germination and plant growth of soybean.

2. Materials and Methods

Seeds were exposed for 30min to ultrasound frequency generated by using Airborne Acoustic (25kHz). The seeds exposed to the ultrasound treatment will be referred to as US while the non-treated seeds will be named NTS.

The plasma reactor used in this work to treat water was a Dielectric Barrier Discharge (DBD) system with a maximum voltage output in the range 0-120 kV_{RMS} at 50 Hz. The plasma discharge was generated at atmospheric air, 80kV voltage and a frequency of 50Hz in alternating current (AC). For generation of PAW, prior to each treatment, a Petri dish containing 20 mL of deionized water was confined into a polypropylene container (310mmx230mmx22mm) and sealed inside a high barrier polypropylene bag to avoid dispersion of volatile chemical species produced during the discharge. Different treatment durations were chosen, 1min and 5min; the samples obtained will be referred to as P1 and P5 respectively.

The chemical characterization of water was carried out by measuring pH, NO_2^-, NO_3^- and H_2O_2.

ATR-FTIR and SEM were carried out on NTS and US treated seeds to identify any chemical and morphological changes induced on the seed surface after the ultrasound

treatment. The seeds interaction with water was evaluated by static WCA and water up-take measurements; moreover, to observe differences in water uptake following ultrasound exposure the NTS and US seeds were dipped for 5 minutes in a fluorescein solution (100µg/mL) and then imaged with an epifluorescence microscope.
Germination of US and NTS seeds watered with untreated deionized water (W) or P1 or P5 was evaluated over 3 days in vitro according to **Equation 1**.

$$G\% = \frac{n^{\circ}\ of\ germinated\ seeds\ in\ each\ Petri\ dish}{total\ n^{\circ}\ of\ seeds\ in\ each\ Petri\ dish} \cdot 100 \qquad (1)$$

The growth in soil of US and NTS seeds watered with untreated deionized water (W) or P1 or P5 was evaluated over 20 days. After 20 days of the soil growth experiment, each plant was extracted from the soil, washed and gently blotted. The roots were removed, and the remaining parts were weighed (Fresh Weight,Wf), then dried in an oven overnight at 102°C and weighed again (Dry Weight,Wd). The moisture content (MC%) was calculated using **Equation 2**.

$$MC\% = \frac{(W_f - W_d)}{W_f} \cdot 100 \qquad (2)$$

3. Results and Discussion

In agreement to previous studies, in this work air plasma treatment of water led to the formation of RONS species and to the decrease of pH. As it can be observed in **Table 1**, longer treatment time resulted in higher concentrations of NO_3^- and H_2O_2, however, NO_2^- was not detected. The influence of plasma treatment on water pH is depicted in **Figure 1**. Plasma treatment drastically reduced water pH in 1 minute from 4.9 ± 0.2 (W) to 3.5 ± 0.2 (P1), while for longer treatment time pH decreased at a slower rate reaching a value of 2.7 ± 0.1 (P5).

Table 1. NO_2^-, NO_3^- and H_2O_2 concentrations (µM) in samples of W, P1 and P5. ND is for non detectable.

	W	*P1*	*P5*
NO_2^-	ND	ND	ND
NO_3^-	ND	20 ± 1	170 ± 9
H_2O_2	ND	20 ± 1	100 ± 5

Water absorption analysis showed that all US samples absorbed more water than the non-treated control seeds. It can be supposed that the interaction between the ultrasound waves and the surface of the seeds is responsible for a higher and easier absorption of water as a result of chemical or morphological modifications. On the other hand, the impact of the water treatment time on the water absorption seems to be irrelevant.

In was observed that airborne ultrasound does not influence hydrophobic properties of the seed coat as no

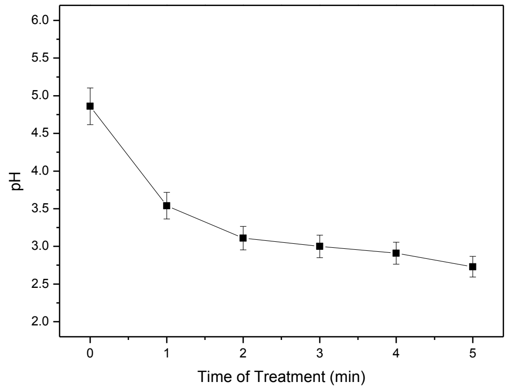

Fig. 1. Variation of pH as a function of time of treatment.

significant differences in WCA values were found for NTS and US using as probing liquid W, P1 and P5. SEM analysis was carried out to study the morphology of the seed coat subjected to 30 min of ultrasound treatment, which demonstrated that seeds were not affected by the treatment. The formation of pores of nanometric dimensions cannot be excluded given the high complexity of the coat surface and the presence of the metallic coating that could mask them.

On the other hand, as it can be observed in **Figure 2**, though FTIR spectra are quite complex, the absorption intensity of the bands at 1318 cm^{-1} and 1022 cm^{-1} clearly decreases upon the ultrasound treatment. It is not easy to attribute these bands to specific chemical bonds, but it is undeniable that a chemical change occurred on the surface of the seed as a result of the contact with ultrasound waves.

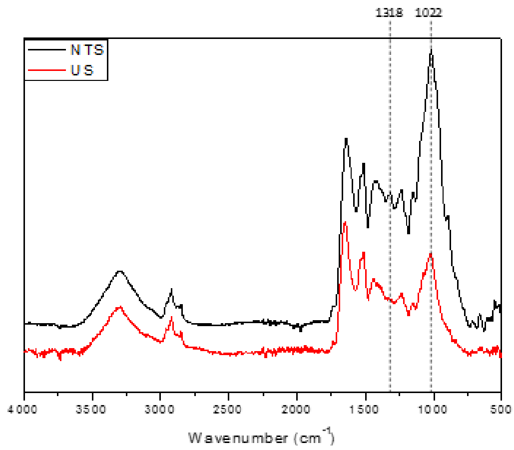

Fig. 2. ATR spectra of NTS and US.

The chemical-morphological results reported so far do not led to well identify the reason for the increased water absorption of US. On the other hand, the fluorescence microscopy images, reported in **Figure 3**, indicate a different path of water absorption for NTS and US. NTS seeds show fluorescence originating almost completely from the coat, with a faint emission from the abaxial parenchyma, suggesting a superficial uptake. The seed section images for US, instead, show that fluorescence originates from the inner part of the cotyledon, in particular from the vascular bundles. Therefore, the fluorescence location is consistent with a deeper, and faster, water uptake in the US samples.

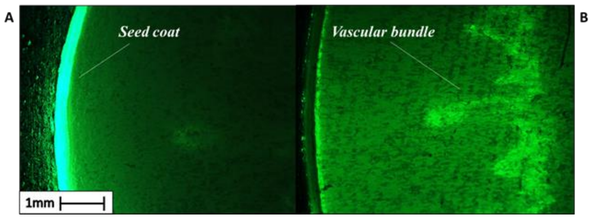

Fig. 3. Fluorescence microscopy images of seed section for a NTS (A) and a US (B).

Germination ratio, reported in **Figure 4**, shows that watering with plasma treated water is effective in enhancing seed germination for untreated seeds, whereas it is not so relevant when the seeds are ultrasonicated. On the other hand, the ultrasound treatment can positively influence the formation of sprouts.

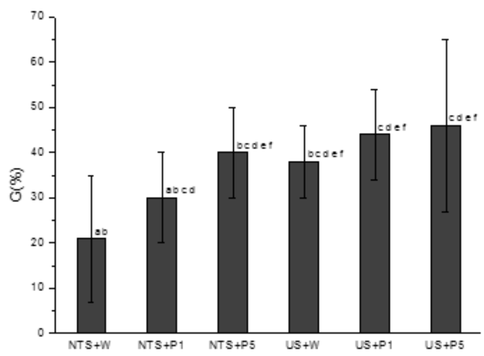

Fig. 4. Values of G % for NTS and US watered with W, P1 and P5 obtained on Day 2.

From in soil growth test it has resulted that the optimum watering option for the growth of NTS is to use P1 while P5, even if more effective then W, seems to be slightly less active. Using P1 is the better option also for watering US but P5 in this case does not differ from W. P5 is more acid so the interaction with the soil can have negative effects on plant growth. At Day 10 the seeds (NTS and US) watered with P1 appear to grow faster, to be healthier and to have a higher germination ratio as illustrated in **Figure 5**.

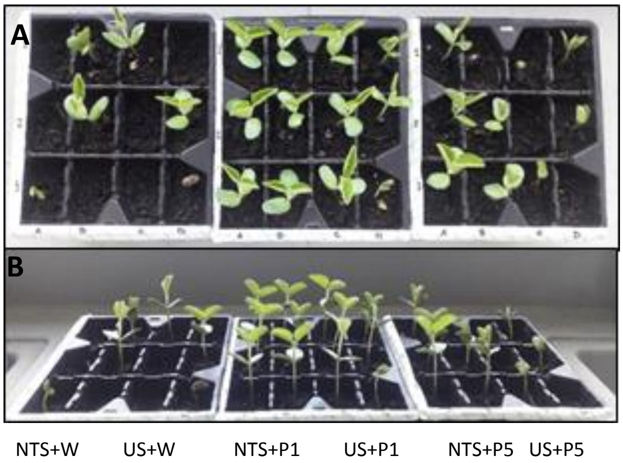

Fig. 5. Images of the plants growing from the seeds (NTS or US) daily watered with W, P1 or P5 10 days after sowing A) from top view and B) from side view.

Plants grown from US watered with PAW have a moisture content statistically higher than the control (NTS+W) and this means a better hydration of the plants.

In conclusion, exposing soybean seeds to airborne ultrasound waves can increase the hydration of the plants and the ability of the seeds to absorb water. When water which was plasma-treated for 1 min (P1) was used for watering the seeds in soil, faster growth and taller soybeans plants are observed after 10 days.

4. References

[1] Puac N., Gherardi M., Shiratani M. (2018) Plasma Process Polym. 15:e1700174.

[2] Misra N.N., Schluter O.K., Cullen P.J. Eds. (2016) Cold Plasma in Food and Agriculture, 1st edition, Academic Press.

[3] Park D.P., Davis K., Gilani S., Alonzo C.-A., Dobrynin D., Friedman G., Fridman A., Rabinovich A., Fridman G. (2013), Cur. Appl. Phys. S19eS29.

[4] Oehmigen K., Hähnel M., Brandenburg R., Wilke Ch., Weltmann K.-D., von Woedtke Th. (2010) Plasma Process. Polym. 7, 250–257.

[5] Lu P., Bohem D., Bourke P., Cullen P.J. (2017) Plasma Process Polym. 14:e1600207

[6] Yang H., Gao J., Yang A., Chen H. (2015) Food Res. Int. 77, 704–710.

[7] Wang Q., Chen G., Yersaiyiti H., Liu Y., Cui J., Wu C., Zhang Y., He X. (2012) PLoS ONE, 7, e47204.

[8] Lopez‑Ribera I., Vicient C.M. (2017) Plant Methods, 13:31.

[9] Tabaru M., Fumino R., Nakamura K. (2015) Acoust. Sci. & Tech. 36, 167-170.

[10] Ghafoor M., Misra N.N., Mahadevan K., Tiwari B.K. (2014) Ultrason. Sonochem. 21, 409-414.

[11] Miano A.C., Forti V.A., Abud H.F., Gomes-Junior F.G., Cicero S.M., Augusto P.E.D. (2015) Seed Sci. & Technol. 43, 2, 1-6.