



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

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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. Different combinations of these two technologies were tested in order to study their interaction and to identify an optimum treatment process. *Industrial relevance:* A great urgency in crop management is to enhance sustainability. The aim is to achieve a cheap and eco-friendly production process reducing the wide current use of energy, irrigation water, chemicals and pesticides. Soybeans is a legume whose worldwide production is increasing in the last years therefore a higher efficiency and sustainability in its cultivation is obviously very appealing. Cold plasma and Ultrasound technologies are well-known in the industrial scenario and their applications in crop production are recently drawing attention; the potential of combining these two powerful techniques is clearly very promising.

1. Introduction

Soybean (*Glycine max*) is a legume that is widely grown for its edible bean, which can be consumed without further processing, defatted to produce a proteic animal feed, treated to produce substitutes for meat and dairy products (such as tofu) and used for the production of fermented products such as soy sauce. In 2015/2016 the worldwide production of soybean was 313.71 million metric tons and an 11.10% increase has been estimated for 2016/2017 (WASDE-573). In light of land use limitations and the need for sustainable intensification of horticulture, there is a need to drive yield increases in important versatile crops such as soybean.

Cold Plasma science and technology has achieved many results since the 1970's, and it is still developing applications, processes and products (Adamovich et al., 2017; d'Agostino, Favia, Oehr, & Wertheimer, 2005). Non-thermal atmospheric-pressure plasma is becoming increasingly important in agriculture (Misra, Schluter, & Cullen,

2016; Puac, Gherardi, & Shiratani, 2018). This technique is suitable for treatment of biological samples, operating approximately at room temperature and pressure. The technology is considered environmentally friendly as systems can be designed to operate with atmospheric air only, and the process does not produce waste. Moreover, in this research field multiple applications have been explored so far, which may be classified into 3 main categories (Ito, Oh, Ohta, & Shiratani, 2018):

- 1- Decontamination of agricultural products
- 2- Enhancement of seed germination and growth of plants, beneficial microorganisms, and worms
- 3- Removal of organic volatile compounds

In particular, the second point can be mainly achieved by direct treatment of seeds (Bormashenko et al., 2015; Bormashenko, Grynyov, Bormashenko, & Drori, 2012; Dobrin, Magureanu, Mandache, & Ionita,

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2015; Filatova et al., 2014; Jiang et al., 2013, 2014; Li et al., 2014; Li, Li, Shen, Zhang, & Dong, 2015; Mitra et al., 2014; Šerá, Šerý, Štraňák, Špatenka, & Tichý, 2009; Šerá, Špatenka, Šerý, Vrchotová, & Hrušková, 2010; Stolárik et al., 2015; Zhuwen, Yanfen, Yanfen, Size, & Wei, 2011) or producing plasma activated water (PAW) used plant irrigation. Sivachandiran and Khacef (2017) studied the combined effect of non-thermal plasma (NTP) treatment of water and seeds on the rate of germination and plants growth of several species of seeds. Direct seed treatment is usually carried out in Dielectric Barrier Discharge (DBD) configuration and can enhance the seed germination by improving the wettability of its surface, increasing the antioxidative activity of cells in response to oxidative stress and other physical and chemical interactions. The production of PAW is achieved by different configurations of plasma including: DBD, gliding arcs, DC, AC or pulsed coronas. 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 (Lu, Bohem, Bourke, & Cullen, 2017; Oehmigen et al., 2010; Park et al., 2013). 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^- . High levels of H_2O_2 can lead to plant cell damage due to its strong oxidizing capacities, but an appropriate amount can have positive effects on the seed germination. These include oxidation of stored proteins causing their mobilization toward the forming axis, crosstalk with other molecules and phytohormones such as abscisic acid (ABA) and gibberellins (GAs), that have a role in the dormancy/germination equilibrium, weakening of the endosperm thereby inducing programmed cell death (PCD) into the aleurone cells thus facilitating its rupture and protection against harmful pathogens (Wojtyła, Lechowska, Kubala, & Garnzczarska, 2016). Moreover, nitrates are fundamental in plants nutrition given their key role in the synthesis of amino-acids, proteins, chlorophyll, etc. Compared to other fertilizers, PAW can be manufactured cheaply from water and air, which along with the absence of organic compounds and pollutants make the technique attractive.

Ultrasound, acoustic waves generated at frequencies higher than 20 kHz, are extensively used in agro-industry. The application in food industry can be divided into low energy (frequencies higher than 100 kHz at intensities below $1 W\cdot cm^{-2}$) used for non-invasive analysis and high energy (frequencies between 20 and 500 kHz and intensities higher than $1 W\cdot cm^{-2}$), which is disruptive and can affect physical, mechanical or chemical/biochemical properties of foods (Awad, Moharram, Shaltout, Asker, & Youssef, 2012). High energy ultrasound is commonly applied in processes, such as the destruction of foams, extraction of flavorings, filtration and drying, mixing and homogenization and many others (Sun, 2014).

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 (Yang, Gao, Yang, & Chen, 2015; Wang et al., 2012; Lopez-Ribera & Vicient, 2017; Tabaru, Fumino, & Nakamura, 2015; Ghafoor, Misra, Mahadevan, & Tiwari, 2014; Miano et al., 2015). The positive effects of ultrasound treatments are due to cavitation, defined as the phenomena of the formation, growth and subsequent collapse of microbubbles, which release large magnitude of energy and induce localized extreme conditions (Wu, Guo, Teh, & Hay, 2013). This phenomenon leads to the formation of pores on the seed surface and subsequently to an acceleration of the rate of influx or uptake of water into the seed. 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. To the

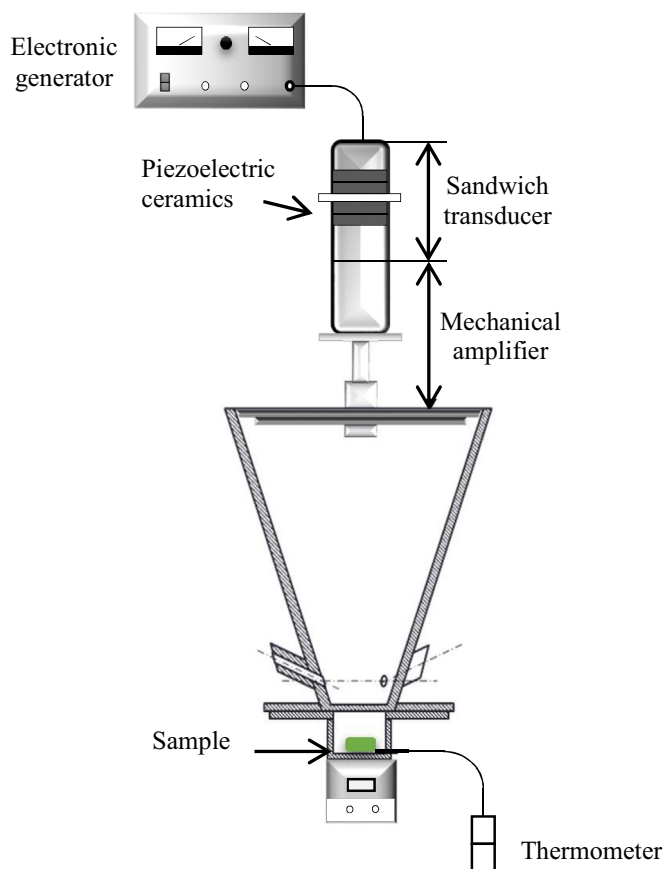


Fig. 1. Scheme of the Airborne Acoustic ultrasound generator.

best of the authors' knowledge, this is the first work reported on the combination of these two promising techniques.

2. Materials and methods

2.1. Ultrasound treatment of seeds

Soybeans (*Glycine max* (L.) Merr) were purchased from a local store. The seeds (180 g) were placed on a metallic mesh and exposed to 30 min of ultrasound treatment generated by using Airborne Acoustic, whose scheme is represented in Fig. 1, at a frequency of 25 kHz. The seeds exposed to the ultrasound treatment will be referred to as US while the non-treated seeds will be named NTS.

2.2. PAW generation

The plasma reactor used in this work was a Dielectric Barrier Discharge (DBD) system with a maximum voltage output in the range 0–120 kV_{RMS} at 50 Hz, described previously by Sarangapani et al. (2016). The apparatus presents two parallel aluminium plate electrodes of circular geometry (outer diameter of 158 mm). Two dielectric layers were placed in contact with the high voltage electrode and the ground electrode with a thickness respectively of 10 mm and 2 mm. The plasma discharge was generated at atmospheric air, 80 kV voltage and a frequency of 50 Hz 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 (310 mm × 230 mm × 22 mm) and sealed inside a high barrier polypropylene bag (Cryovac, B2630, USA), to avoid dispersion of volatile chemical species produced during the discharge. The package containing the samples was placed directly in contact with the dielectric layers, in the plasma zone, and treated for either 1 min or 5 min (P1 and

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