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The effect of non-thermal plasma treatment on wheat germination and early growth

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ABSTRACT

The influence of non-thermal plasma treatment on wheat seeds (*Triticum aestivum*) has been investigated using a surface discharge reactor at atmospheric pressure and room temperature. Growth parameters, like roots and sprouts length and dry weight were measured on the fourth day of germination and a Gaussian distribution was used for curve-fitting of the obtained results. It was found that plasma had little effect on the germination rate, but influenced growth parameters. In the case of plasma treated seeds, the distribution of roots was shifted towards higher lengths as compared with the untreated samples. The distribution of the sprouts length was about two times narrower for the treated samples as compared with the control seeds. The sprouts and roots of the plasma treated seeds were heavier than those of the control samples. The root-to-shoot (R/S) ratio differed substantially, being 0.88 ± 0.016 for the untreated seeds and reaching 1.2 ± 0.005 for the treated samples.

Industrial relevance: The results obtained in this research demonstrate that non-thermal plasma treatment has a positive effect on wheat early growth. Due to its advantages (uniform treatment, no destruction of seeds, no requirement for chemicals), plasma might become an effective alternative to traditional pre-sowing seed treatment used in agriculture.

1. Introduction

Non-thermal plasma has been recently investigated in the field of agricultural science as an alternative to the traditional pre-sowing seed treatment, such as physical scratching (scarification), heat treatment, and chemical treatment (Dhayal, Lee, & Park, 2006). For physical scratching, the seed coat is scratched using a grain feed-mixer at minimal cost. However, this mechanical procedure has a high probability of increasing the number of destroyed seeds as well as non-uniform treatment. In the case of heat treatment, hot water or a hot surface is used, while the chemical treatment consists in coating the seed with commercial sulphuric acid for 5–10 min before washing in water and drying, but this process results in environmental pollution (Dhayal et al., 2006). Plasma has the advantage of uniform treatment, there is no destruction of seeds, and plasma does not require chemicals, hence it is harmless for the environment (Dhayal et al., 2006; Selcuk, Oksuz, & Basaran, 2008; Volin, Denes, Young, & Park, 2000).

Recently published works indicate the positive effect of plasma treatment on seed germination and on plant development and growth (Dhayal et al., 2006; Kitazaki, Koga, Shiratani, & Hayashi, 2012; Lynikiene, Pozeliene, & Rutkauskas, 2006; Sera, Sery, Stranak, Spatenka, & Tichy, 2009; Sera, Spatenka, Sery, Vrchotova, & Hruskova, 2010; Sera, Stranak, Sery, Tichy, & Spatenka, 2008). Changes in enzyme activity of plants as a result of exposure of seeds to plasma have also been reported (Henselova, Slovakova, Martinka, & Zaharanova, 2012). One consequence of the plasma treatment is the sterilization of the seed surface (Basaran & Akhan, 2010; Basaran, Basaran-Akgul, & Oksuz, 2008; Selcuk et al., 2008). It was found that seed germination can be tuned by plasma chemistry; more precisely the germination can be delayed or accelerated without significantly affecting their germination rate, by creating a plasma-reacted deposit on the surface of seeds (Volin et al., 2000).

An important part of the investigations reported on seeds treatment were carried out using low-pressure radio frequency (RF) discharges (Bormashenko, Grynyov, Bormashenko, & Drori, 2012; Dhayal et al., 2006; Filatova et al., 2011; Filatova et al., 2013; Kitazaki et al., 2012; Puac et al., 2005; Volin et al., 2000; Zivkovic, Puac, Giba, Grubisic, & Petrovic, 2004) and microwave discharges (Basaran & Akhan, 2010; Sera et al., 2008; Sera et al., 2009; Sera et al., 2010). Some studies with atmospheric pressure discharges, such as corona (Lynikiene et al., 2006) and diffuse coplanar barrier discharge (Henselova et al., 2012) have been published as well. The authors investigated the germination of various types of seeds: safflower (Dhayal et al., 2006), radish (Kitazaki et al., 2012; Lynikiene et al., 2006; Volin et al., 2000), lamb's quarters (Sera et al., 2008; Sera et al., 2009), oat (Sera et al., 2010), wheat (Bormashenko et al., 2012; Filatova et al., 2013; Sera et al., 2010), maize (Filatova et al., 2013; Henselova et al., 2012), blue lupine

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(Filatova et al., 2011; Filatova et al., 2013), *Paulownia tomentosa* (Puac et al., 2005; Zivkovic et al., 2004) etc.

An increase in the germination rate of the plasma treated seeds as compared with the control (untreated seeds) was reported by several authors (Dhayal et al., 2006; Lynikiene et al., 2006; Puac et al., 2005; Sera et al., 2008; Zivkovic et al., 2004). For example, Dhayal et al. (2006) obtained a 50% higher germination rate of safflower (*Carthamus tinctorium* L.) seeds exposed to RF plasma generated in argon for 130 min. Sera et al. (2008) obtained almost a three time increase of the germination rate of the lamb's quarters (*Chenopodium album*) after treatment with a microwave discharge for 48 min. In contrast, other studies reported no influence of plasma on the germination rate of wheat (Selcuk et al., 2008), bean (Selcuk et al., 2008), oat (Sera et al., 2010) or radish (Kitazaki et al., 2012). In some cases, improved results as compared with the untreated samples were obtained for optimized plasma treatment times (Dhayal et al., 2006; Filatova et al., 2013; Henselova et al., 2012; Puac et al., 2005; Sera et al., 2008; Zivkovic et al., 2004).

'Several authors reported enhanced growth parameters of seeds after plasma exposure (Dhayal et al., 2006; Henselova et al., 2012; Kitazaki et al., 2012; Sera et al., 2008). Sera et al. (2008) reported 50% increase in sprouts length of the lamb's quarters after treatment in a microwave discharge. An increase with 60% in the sprout lengths of radish exposed to oxygen RF plasma was reported by Kitazaki et al. (2012). Henselova et al. (2012) reported 21% increase in length, 10% increase in fresh weight and 14% increase in dry weight of maize roots treated in a diffuse coplanar surface barrier discharge generated in ambient air. Two times higher length of safflower roots treated by argon RF plasma was obtained by Dhayal et al. (2006).

In this work the influence of treatment in a surface discharge generated in air at atmospheric pressure and room temperature on wheat seeds was investigated for the first time. The germination rate and growth characteristics, such as roots and sprouts length and dry weight were determined. The modification of wetting properties of seeds by plasma treatment was also investigated.

2. Materials and methods

2.1. Plasma reactor and electrical circuit

The seeds were treated by non-thermal plasma using a surface discharge reactor, at atmospheric pressure and room temperature. The set-up is illustrated in Fig. 1.

The plasma reactor consists of two electrodes placed on both sides of a glass plate (length 120 mm, width 90 mm, thickness 1.5 mm). The high voltage electrode is an array of 13 copper wires, each wire having a diameter of 100 μm and length of 44 mm. The distance between adjacent wires was 6 mm. The ground electrode was an aluminium tape (length 90 mm, width 48 mm). The seeds were distributed uniformly on each wire. The plasma reactor was placed in a rectangular case and air was flown with a rate of 1 L/min.

The discharge was generated in a.c. mode using a high voltage transformer (TIRB 0–20, Electroputere, Romania, transformation ratio 300),

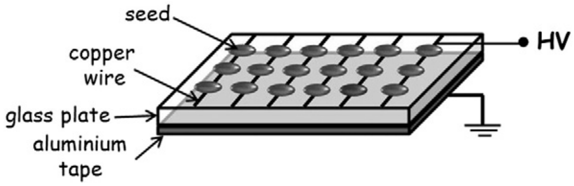

Fig. 1. Experimental set-up: HV — a.c. high voltage transformer, high voltage electrode — array of 13 copper wires (diameter 100 μm, length 44 mm, distance between adjacent wires 6 mm), ground electrode — aluminium tape (length 90 mm, width 48 mm), glass plate (length 120 mm, width 90 mm, thickness 1.5 mm), and seeds — *Triticum aestivum* (private collection).

which provides sinusoidal voltage at 50 Hz frequency. The discharge voltage was measured by a high voltage probe (P6015A, Tektronix, USA). The current was measured with a shunt resistor of 3 Ω connected in a series with the ground electrode. The total charge dissipated in the discharge was measured with a non-inductive capacitor of 1.5 μF placed instead of the shunt resistor. The discharge characteristics were monitored by a digital oscilloscope (DPO 2024, Tektronix, USA). The average electrical power dissipated in the discharge was calculated by the Lissajous method (Falkenstein & Coogan, 1997). The Lissajous figure, obtained by plotting the total charge versus applied voltage, is a parallelogram, whose area is equal to the energy deposited in the discharge in a cycle (Falkenstein & Coogan, 1997). The average power dissipated in the discharge was calculated by multiplying this area with the frequency of the applied voltage.

2.2. Seed material

Wheat seeds (*Triticum aestivum*) were chosen for this study, since wheat represents one of the most important crop plants all over the world. The seeds were obtained from a private collection. They were visually scanned, and only healthy seeds without visible defects were selected for treatment. Two seed lots were prepared, each lot containing 105 seeds. The seeds from one lot were exposed to plasma, while the seeds from the other lot were used as control. The seeds to be treated were distributed uniformly on the wires in such a way that individual seeds did not touch each other and exposed to plasma for a duration of 5, 15, 30 min.

Plasma treated and control seeds were placed into Petri dishes (90 mm diameter) containing one layer of filter-paper impregnated with 5 ml distilled water. Each Petri dish contained 15 seeds. The dishes were covered with lids and wrapped using a strip of parafilm to prevent evaporation of water. Wheat seeds were cultivated in an incubator at 22 °C, in the dark for four days.

The germination rate (defined as the number of germinated seeds divided by the total number of seeds) and growth parameters (average length of roots and sprouts) were measured in the fourth day of germination. After the measurements, roots and sprouts were dried under laboratory conditions until they reached a constant mass and then were weighted using an analytical balance (AS 220/C/2, Radwag, Poland). The root-to-shoot ratio (defined as the ratio between the dry weight of roots and dry weight of sprouts) was also determined.

For the study of the time dependence of water absorption by wheat seeds, 45 untreated and 45 plasma treated (for 15 min) seeds were placed into Petri dishes. Each dish contained 15 seeds placed on one layer of filter paper impregnated with 5 ml of distilled water. The seeds were weighted every 30 min in the first 90 min of experiment, and then every 60 min until 390 min.

Contact angle measurements were performed by an optical system (CAM 101, KSV Instruments, Finland) based on capture and automatic analysis of drop shape images for measuring contact angles. The instrument is equipped with a FireWire digital CCD camera, a LED light source, and a manual Hamilton precision syringe. The high resolution CCD camera ensures that the images are captured and transferred to the software without distortion, then image analysis is performed on the image stored on the computer. The contact angle is determined from the Young equation, which considers thermodynamic equilibrium between the solid phase S, the liquid phase L and the gas/vapour phase G:

$$\gamma_{SG} - \gamma_{SL} - \gamma_{LG} \cdot \cos\theta_C = 0,$$

where θ_C is the contact angle, γ_{SG} is the solid–vapour interfacial energy, γ_{SL} is the solid–liquid interfacial energy and γ_{LG} is the liquid–vapour interfacial energy.

In the present experiments, the contact angle between the surface of the seeds and liquid (in our case distilled water) has been measured at room temperature by placing a drop of 1 μl of liquid on the surface.