**ORIGINAL PAPER** 



# Non-thermal Plasma as a Priming Tool to Improve the Yield of Pea in Outdoor Conditions

Gervais B. Ndiffo Yemeli<sup>1</sup> · Mário Janda<sup>1</sup> · Zdenko Machala<sup>1</sup>

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#### Abstract

Seed priming is a pre-treatment of seeds leading to the improvement of their germination, the plant growth, and the product yield. In this study we investigated the possibility of the use of non-thermal plasma operating in atmospheric pressure air for seed priming with the objective to improve the yield of pea seeds. Two priming ways were used: an indirect way by using plasma activated water (PAW) generated by the transient spark discharge with water electrospray or the glow discharge batch treatment and a direct exposure of seeds to the pulsed corona discharge. After treatment, the seeds were planted in the outdoor field for about 14 weeks until harvest. The direct plasma treatment resulted in two key results: the strong effectiveness of the pulsed corona plasma improving the yield, and the long-term effect of the plasma seed treatment. The results of the indirect treatment showed that the pea plants from the seeds primed using PAW gained some improved growth parameters, especially the number of seeds per pod and the total number of seeds per plant. The scanning electron microscopy analysis showed that PAW and direct treatment induced some morphology changes at the surface of the pea seeds. This study documents a long-term effect of non-thermal plasma seed priming and contributes to the plasma agriculture applications by suggesting the implementation of non-thermal plasma direct or indirect treatments into the field.

**Keywords** Non-thermal plasma · Direct and indirect treatment · Pea · Agriculture · Yield · Plasma activated water · Seed priming

# Introduction

Reaching the global objective of providing enough food for people by increasing the crop yield, while reducing the environmental pollution from the use of agricultural chemicals (herbicides, pesticides, ...), became one of the key elements of the sustainable agriculture

Gervais B. Ndiffo Yemeli gbn.yemeli@fmph.uniba.sk

Zdenko Machala machala@fmph.uniba.sk

<sup>&</sup>lt;sup>1</sup> Division of Environmental Physics, Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Mlynská Dolina, 842 48 Bratislava, Slovakia

research. The Food and Agriculture Organisation (FAO) is questioning the researchers if in 2050, there will be enough land, water, and other natural resources to produce food and feed the increasing world population [1]. This questioning has already been a source of concern and continues to be so, and leads the researchers to find new approaches to improve the yield by reducing the use of products harmful for ecosystems (soil, surface and underground water, etc.). One of the new approaches is the seed priming, which is a pre-treatment of seeds either in wet or dry way with the aim to improve their stress tolerance, to improve their germination, to enhance the plant growth, and to improve the product yield [2–5]. The seed priming is considered as an advantageous agricultural and economic method to protect the environment and restore the damaged/degraded soil [2–4, 6–9].

Many approaches are currently used for the seed priming: e.g. the halo treatment, the hydro treatment, the physical treatment, and the chemical treatment [4, 5, 10-12]. The effectiveness of these approaches depends on several factors, such as the priming agent, temperature, priming time, light, seed humidity, seed family [2, 6, 8, 13].

The physical treatment which is less frequently used, involves the use of UV radiation, gamma radiation, X-rays, or cold, applied on the seeds [2, 14]. Shine et al. [15] showed that by applying different magnetic field strengths on soybean seeds, they obtained higher germination parameters, such as germination speed, seedling length, fresh weight and water uptake compared to control. Bukhari et al. [16] showed that the effectiveness of the magnetic field applied on hybrid sunflower seeds on growing parameters and antioxidant activities depends on the magnetic field strength and exposure time. In the same physical approach for seed priming, Talei et al. [17] exposed rice seeds to microwave radiation at the frequency of 2450 MHz for 1, 4 and 10 h. The 10 h exposure showed a better germination percentage and improved the germination indices.

Other conventional priming methods are based on the imbibition of seeds in liquid medium/aqueous solution. The hydro-priming method is a simple, economical technique and is based on soaking of the seeds in water for a determined time. This method has been used by several authors to improve the growth parameters [18–22]. Besides hydro-priming, the chemical priming is also well used, and consists of a treatment/soaking of the seeds with chemical products, such as hydrogen peroxide or urea. The positive impact of this method has been shown in numerous articles, on maize [23–25], on winter wheat [7], on rice [26, 27], on pea [28]. The halo-priming method which uses soaking the seeds in salt solutions, such as NaCl, KNO<sub>3</sub>, has also shown some positives impact on the seed germination, seedlings enhancement, seed growth under drought and salt stress [23, 29–31]. Although many of these methods have shown positive effect on seed growth and enhancement, non-thermal plasma (NTP) was also identified as an alternative priming source for seeds.

NTP is an efficient source of radicals, electric field, and reactive species often coupled with the effects of heat and UV radiation [32]. Due to these properties, direct seed treatment by the NTP can be considered as the physical priming method (due to UV radiation, electric field, and heat). Once the plasma in contact with water through the water/gas interface, it induces chemical changes in the treated water by producing the reactive oxygen and nitrogen species (RONS) and transporting them into the water [33, 34]. The resulting water is called plasma activated water (PAW). This water contains some long-lifetime species such as hydrogen peroxide, nitrate, and nitrite ions. The PAW or indirect plasma seed treatment can be assigned among the chemical priming (due to hydrogen peroxide) and halopriming (due to nitrate and nitrate ions) methods. Since 2010, the number of published articles related to the use of NTP (direct or indirect) methods for seed priming increased due to the relatively promising results obtained so far. The published articles using the

PAW are not so well represented, nevertheless some authors showed good and promising effects. Rathore et al. [35] showed that when they primed pea seeds for 24 h in PAW generated by pencil plasma jet, they obtained better cumulative germination (37% and 26% for PAW 5 min and P 10 min, respectively) and plant growth parameters (an improvement for the root length of 26% and 38%, respectively, for PAW 5 min and PAW 10 min, and for 57% and 95% for shoot length, respectively, PAW 5 min and PAW 10 min) compared to control primed in pure water. They also obtained higher protein and sugar concentration compared to control. Alves junior et al. [36] also reported that by soaking Erythrina velutina seeds in PAW generated by dielectric barrier discharge (DBD), the vigor and the and cumulative germination rate (an average of 20% for seed treated with PAW) were better compared to the ones soaked in the untreated water. Terebun et al. [37] showed that, the PAW generated by gliding arc discharge (GAD) operating in air as working gas enhance the germination rate of Beta vulgaris seeds (after 7 days, germination energy GEN showed a germination boost of 17%, 17% and 22%, respectively, for PAW 5 min, PAW 10 min and PAW 20 min compared to control distilled water). Regarding the direct treatment, several authors published results showing the positive effect of using the NTP for seed priming. For example Pawlat et al. [38] reported some positive effect on the germination of Thuringian Mallow seeds treated by gliding arc discharge operating at atmospheric pressure. They showed that the Thuringian Mallow seeds pre-treated by plasma for 1, 2, 5, 10 and 15 min induced a boost of germination energy (G<sub>EN</sub>) after 10 days of the order of 25%, 45%, 42%, 28% and 30%, respectively, compared to the untreated control. They also reported an enhancement of the germination capacity ( $G_{C}$ ) after 21 days of the order of 17%, 40%, 40%, 13% and 15%, respectively, for 1, 2, 5, 10 and 15 min compared to control. Jiang et al. [39] showed that the treatment of wheat seeds by radiofrequency plasma induced an improvement for the germination potential (6%) and germination rate (6.7%) compared to control. Regarding the field experiments, the wheat seeds treated by radiofrequency plasma at 80 W showed an improvement of the yield (5.89%) compared to control. At the same time the plant height (21%), fresh weight (7%), and stem diameter (9%) were improved at the booting stage for the plasma seed priming compared to control. The 80 W radio frequency plasma priming effect was also tested on wheat, the seeds treated at 180 s showed positive effect by increasing the grain yield compared to control [40]. Brust et al. [41] also showed some positive germination effect of the DBD treatment of wheat and barley seeds compared to control. They also reported that even after 1 and 2 months of storage, both seed families treated by DBD still germinate faster compared to the control. Several other articles also reported some positive impacts by using NTP seed priming for the improvement of the plant growth parameters [42–51].

These positive impacts of NTP (indirect and direct way) for seed priming showed by several authors for the improvement of the germination, the enhancement of seed growth, and the yield open a wide area and a need to be further explored. Especially the effects of PAW priming on the final product yield are of the greatest interest, since no articles have investigated the priming effect of PAW on the final yield.

Following this idea, the aim of this work is to present a novel approach of the of use of the NTP seed priming (direct and indirect treatment) to improve the final yield of pea (*Pisum sativum* L.) cv. Eso. The study is carried out using several plasma sources and organized as follows: (1) for the indirect treatment of NTP priming on pea seeds, the transient spark discharge and glow discharge operating at room temperature and atmospheric pressure were used to generate PAW employed for the 24 h seed soaking. (2) direct treatment of pea seeds with the pulsed corona plasma was used. The scanning electron microscopy (SEM) was performed for both treatments to evaluate the changing surface morphology of pea seeds. Before priming experiments, in vivo test was performed to determine the adequate direct plasma treatment time for the field test. Then the pea seeds were treated by direct plasma or PAW for the field test. We performed the optical UV–Vis absorption measurements to determine the composition of the pulsed corona plasma gas products (detectable molecules  $O_3$ ,  $NO_2$ ,  $HNO_2$ , NO). Field tests were carried out for both plasma treatments of seeds (direct and indirect), using just rain or tap water for watering. In the end of the 14-week experiment we evaluated the number of pods per plant, the number of seed per pod, the total number of pods per plant, the average pod weight per plant, the average seed weight per plant and the dry weight per plant.

# **Materials and Methods**

## **Target Seed**

Dried pea seeds (*Pisum sativum* L.) cv. Eso, used as target material in the experiments, were obtained from the Slovenské farmárske družstvo, Slovakia.

## Non-thermal Plasma Experimental Setup

## **Indirect Priming**

The seeds were primed by indirect NTP using the plasma activated water generated by transient spark (TS) discharge with water electrospray and glow discharge (GD) with water cathode, both operating at atmospheric pressure and ambient air. The detailed plasma setup was presented in our previous articles [52, 53]. A high voltage DC power supply at positive polarity was used for both plasma discharges (Umax = 20 kV, Imax = 30 mA, Pmax = 600 W). DC high voltage is applied through the ballast resistors (8.8 MΩ for TS and 0.5 MΩ for GD) on the high voltage electrode (4 stainless steel needles for TS and 1 needle for GD). The PAW used in this research was prepared from tap water with a conductivity ~450 mS/cm and pH~7.8. For the GD, PAW generation was performed for 1 or 2 min of plasma treatment of 10 mL of tap water. For TS we operated at the constant flow rate 0.5 mL/min until reaching a sufficient volume (usually 50 ml).

## **Direct Priming**

The commercial NTP used for the direct pea seed treatment is a pulsed corona discharge generated by the plasma source DH 1010 (available from Kamea Electronics, s.r.o., Slovakia). The reactor is constituted of two steel plates as electrodes with the high voltage electrode with an array of pins and the ground electrode consisting of a flat plate, as shown in Fig. 1. The complete description of this plasma source operation is provided by Sarangapani et al. [54]. The pulsed corona discharge used to carry out pea seed priming operated with the following parameters: frequency: 3 kHz, duty cycle 54  $\mu$ S, input voltage 225 V, electrode separation 4.5 cm.



Fig. 1 Pulsed corona discharge plasma generated between the multipin and plate electrodes applied for pea seed priming

# **Pulsed Corona Plasma Diagnostics**

Electrical and optical diagnostics of the pulsed corona plasma was performed. Figure 2 shows a schematic of the experimental setup used for electrical diagnostics of the used plasma source. During the experiments focused on characterization of the plasma source, we varied the gap size (2.5–4.5 cm), the generator input voltage (225–265 V), pulse frequency (1–3 kHz), duty cycle (36–108  $\mu$ s) and exhaust gas suction flow rate. The reactor operated in open ambient air, and it was not enclosed in a box. The treated air containing ozone was therefore removed from the laboratory by the suction system with a controllable gas flow rate (6 levels, 0.06–0.16 m<sup>3</sup>/s). The end of the suction pipe was at the same horizontal level as the reactor active zone in a distance about 30 cm.

The electrical current was measured by Rogowski type current monitor (Ion Physics Corp., model CM-500-L, 75 MHz, 5 V/1A). The current was recorded by the digital oscilloscope Tektronix TBS 2000 (4 channels, 100 MHz, 1 GS/s). The position of the current monitor varied during experiments to record the characteristic waveforms of the current flowing in all cables in the circuit: the two feeding cables and the grounding cable (see Fig. 2). The relative voltage was measured by the high voltage probe (Tektronix P6015A, 75 MHz) with its tip approximately 5 cm from the high voltage needle



electrodes of the power source. The HV probe was not attached directly to the electrodes because it is not designed to withstand a voltage above 20 kV. We did not observe any influence of the air suction on the discharge generation and on the measured electrical characteristics.

Figure 3 shows a simplified schematic of the experimental setup used for optical diagnostic of the generated pulsed corona plasma. The UV–Vis absorption spectroscopy was used for the quantitative detection of ozone (Fig. 3a). As a light source, deuterium lamp Avantes AvaLight-D-S was used, with the spectrometer Avantes AvaSpec-Mini4096 CL for spectrally resolved light detection. Spectra in the range 190–650 nm with the spectral resolution is 0.4–0.5 nm were recorded.

Radiation from a deuterium lamp was reflected by a parabolic mirror, which turned it into a parallel beam (diameter ~ 10 mm) that travelled across the plasma at two vertical positions, either ~ 1 cm above the grounded electrode, or ~ 1 cm below the HV needle electrodes. At the other side of the reactor, transmitted part of the radiation entered the optical fibre connected to the entrance slit of the spectrometer. By comparing the spectrum measured with the plasma on and off, we determined the ozone concentration by fitting the measured spectra with the synthetic spectra calculated for 20 cm absorption path. The UV–Vis absorption cross section of  $O_3$  was downloaded from the MPI-Mainz UV/VIS Spectral Atlas [55]. The emission from the plasma was also recorded by the optical spectrometer (Fig. 3b). The light was collected from the area right below the tip of the HV needle electrode. The plasma emission spectra can be used to identify excited species, or to estimate the gas temperature in plasma [56]. The measured experimental spectra were compared with synthetic spectra created by Specair software [57].

#### Scanning Electron Microscopy (SEM)

After the treatment with plasma (dry and wet treatment), high resolution Apreo 2S (Thermo Fischer Scientific) scanning electron microscope was used to analyse the seed surface morphology. The seeds were dried and deposited with a gold conductive layer. The SEM images were taken using 2 kV voltage with  $1000 \times \text{and} 10,000 \times \text{magnifications}$ .



Fig. 3 Simplified schematic of the experimental setup used for optical diagnostics of the pulsed corona plasma source (top views): **a** UV–Vis absorption spectroscopy for ozone detection, **b** optical emission spectroscopy of the plasma

#### **Plasma Seed Priming and Plant Growth Procedure**

#### Indirect Seed Priming

The PAW used for indirect seed priming, prepared by transient spark with water electrospray and by the glow discharge with water electrode, have the same parameters and concentrations of reactive species as shown in our previous article [53]. The concentrations of the long-life species evaluated in the PAW used were 0.3 mM, 0.6 mM, and 1.6 mM, respectively, for  $H_2O_2$ ,  $NO_2^-$ , and  $NO_3^-$  for transient spark (TS, water electrospray flow rate 0.5 mL/min). The  $H_2O_2$ ,  $NO_2^-$ , and  $NO_3^-$  concentrations were 0.9 mM, 0.7 mM, 1.3 mM and 1.4 mM, 0.8 mM, 2 mM, respectively, for glow discharge 1 min treatment (GD 1) and glow discharge 2 min treatment (GD 2). The fresh produced plasma activated water (TS 0.5, GD 1 and GD 2) from both plasma sources was collected, and 50 pea seeds were introduced in the PAW and soaked for 24 h before being sowed into the field. The same number of seeds was used for the control (C) tap water (untreated water) and also for dilute 2 mM HNO<sub>3</sub> solution (Nitric) prepared using tap water, as the nitrate ion source for the 24 h soaking.

The field experiments were carried out in the outdoor field repeatedly from March to June/July in 2020 and 2021. These tests were carried out up to the product yield, after 24 h of priming with PAW. The seeds from indirect PAW treatment were directly transferred into the soil of the outdoor field and the plant growing process started. During the growing, the plants received either groundwater or rainwater irrigation. After the harvest we evaluated the following basic parameters, the number of pea pods per plant, the number of seeds per pod, the average pod weight (g), the average seed weight (mg), the total number of seed per plant, the total pod weight per plant (g).

## **Direct Seed Priming**

A study of the effect of the pulsed corona plasma priming was done on pea seeds. Early germination test was first performed to help us to select the appropriate seed treatment time for the field experiment. For that, 100 pea seeds were treated by pulsed corona discharge for 1, 3, 5 and 10 min (PC-1, PC-3, PC-5 and PC-10, respectively) with the following parameters: frequency: 3 kHz, duty cycle 54  $\mu$ S, input voltage 225 V, electrodes separation 4.5 cm. After the plasma treatment, each variant of treated pea seeds and untreated control (C) were placed in Petri dishes containing 2 layers of filter papers moisturized with tap water for 8 days. Three repetitions of the early germination test were performed for statistical weight. After 8 of germination and early growth for the pulsed corona plasma priming pre-test, we evaluated the shoot and root length (cm), the fresh and dry weight (mg) of shoot and root of the young seedlings.

After evaluating this pre-test, pulsed corona plasma treatment for 3 and 5 min were selected for the field test. For the field test, 100 seeds were primed by pulsed corona plasma for 3 and 5 min; after the treatment the seeds were stored for 6 weeks. After 6 weeks of storage, the unprimed (untreated) and primed pea seeds were soaked overnight for 8 h in tap water. The field experiment was carried out up to the product yield from March to June/July 2021/2022.

In the field test, after 8 h of soaking in tap water after the direct treatment, the seeds from both treatments were directly transferred into the soil of the outdoor field and the



Fig. 4 Experimental field of: a point A and b point B



Fig. 5 Schematic representation of the experimental procedure of seed priming and following field test. a indirect PAW seed priming and b direct plasma seed priming

plant growing process started. During the growing, the plants received either groundwater or rainwater irrigation. After the harvest we evaluated the following basic parameters, the number of pea pods per plant, the number of seeds per pod, the average pod weight (g), the average seed weight (mg), the total number of seed per plant, the total pod weight per plant (g).

The experimental fields were established at two different points, namely A and B. The point A for year 2020 (indirect priming) and year 2021 (both indirect and direct priming) was in the garden of one of the authors. Point B was an approximately 4 square meters field provided by a colleague (indirect priming, year 2021). The point B in 2021 was planned to for testing whether the effect is reproduceable in another field. The soil at the point A was slightly enriched with compost, whereas the point B soil was a sandy soil. No other fertilizers were used. Figure 4 shows the experimental field at the points A and B.

The following picture (Fig. 5) shows the graphical representation of the field test for the PAW and the pulsed corona plasma priming of pea seeds from the priming to the harvest.



Fig.6 Long **a** and short **b** time-scale waveforms of voltage and current of the pulsed corona discharge measured on the grounding cable, power supply settings: frequency 1 kHz, duty cycle 54  $\mu$ s, input voltage 250 V

# **Results and Discussion**

#### **Pulsed Corona Discharge Diagnostics**

Figure 6 shows typical long time-scale waveforms of the measured voltage and current, with the current monitor placed on the grounding wire. Figure 6a shows (at least partly) three applied voltage pulses that could be described as amplitude-modulated oscillating voltage packages. First, the increasing amplitude of voltage oscillations increases (reaching maximum in  $t \sim 70 \ \mu s$  in Fig. 6b), followed by an exponential decrease of the amplitude (like in a case of damped oscillator). The measured current consists of the capacitive displacement current caused by changes of the applied voltage and the real discharge current, i.e. the current attributed to the movement of charge carriers (mostly electrons) in the plasma. The discharge current is manifested as the current peaks (indicated by stars in Fig. 6b) observed when the oscillating voltage reaches peak values. Figure 7 shows a short time-scale waveform of the voltage and the current, where one of the discharge current peaks can be seen in time 0–0.5  $\mu$ s. It is also worth mentioning that these relatively strong current pulses are accompanied by a partial voltage drop. The voltage drop indicates the increase of plasma conductivity and thus increase of electron density, because the conductivity of weakly ionized plasma is directly proportional to the electron density.

The amplitude of the observed current peaks is relatively high (~A), but this is still not a spark current pulse because no gas breakdown occurs, as indicated by only a partial voltage decrease. The spark current pulses with even higher amplitude would appear only as the consequence of the gas breakdown with formation of a highly conductive plasma channel connecting a needle electrode with the grounded one with a strong spark current (>10 A), leading to the voltage drop to zero.

We assume that the high amplitude of the current pulses is due to a combined current from streamer pulses from multiple needle HV electrodes. The streamers are important for plasma induced chemistry, because the reduced electric field strength is very high in the streamer's head (> 200 kV/cm [58, 59]). In such a strong field, electrons can reach high energy and the collisions between high energy electrons and neutral molecules lead to their excitation, dissociation and ionization.

In air, high energy electrons collide mostly with molecules of  $N_2$  and  $O_2$ . These collisions lead to the formation of excited  $N_2$  molecules, such as  $N_2(C^3)$ ,  $N_2(B^3)$ ,  $N_2(A^3)$ ,



Fig. 7 Short time scale waveforms of voltage and current of the pulsed corona measured on the grounding cable, power supply settings: frequency 1 kHz, duty cycle 72  $\mu$ s, input voltage 250 V. Focus on the discharge current pulse (time 0–0.5  $\mu$ s)



**Fig.8** Typical optical emission spectrum of the pulsed corona discharge, frequency 1 kHz, duty cycle 54  $\mu$ s, input voltage 250 V, gap 2.5 cm, measured right below needle electrode tip, the fastest gas flow rate (0.16 m<sup>3</sup>/s). Inlet layer—comparison of the measured spectrum with the synthetic spectrum calculated by Specair for rotational and translational temperatures 300 K

or  $N_2(a^1)$  [60]. Figure 8 shows the optical emission spectrum from the generated pulsed corona plasma, where the second positive system (SPS) associated to the deexcitation of  $N_2(C^3)$  species dominates. By comparing the measured spectra with the synthetic spectra generated by Specair software [57], we estimated the rotational temperature of  $N_2(C^3)$  species to be around  $300 \pm 25$  K (Fig. 8, inset layer). Assuming the translational temperature to be equalized with the rotational temperature at atmospheric pressure, the generated plasma leads to negligible gas heating. The measured temperature close to the laboratory temperature ( $300 \pm 25$  K) is typical for streamer discharges. It is consistent with our previous experiments with streamer corona discharge e.g. [61]. Unlike in the work of Sarangapani et al. [54], the first negative emission system of  $N_2^+$  ion and the emission of OH (A–X) system were very weak in our pulsed corona experiments.

The collisions of electrons with molecular oxygen lead to the production of atomic oxygen either by dissociative excitation, or by electron attachment. The reactions of  $O_2$  with excited  $N_2$  molecules, such as reaction

$$O_2 + N_2(C^3) \rightarrow N_2 + O + O,$$
 (1)

also significantly contribute to generation of atomic oxygen. Subsequently, ozone is generated as the main stable product of the pulsed corona discharge by the reaction

$$O_2 + O + M \rightarrow O_3 + M. \tag{2}$$

Other products, such as nitrogen oxides could be also produced by O atoms, but their formation at 300 K is very slow. We observed no nitrogen oxides (NO or NO<sub>2</sub>) in UV–Vis absorption spectra. Based on the noise level and the sensitivity of our system, the concentration of nitrogen oxides was certainly below 5 ppm. We only observed absorption by ozone, reaching concentrations in the range 30–150 ppm, depending on air flow rate and plasma source settings.

Figure 9 shows a typical UV–Vis absorption spectrum measured ~1 cm above the planar grounded electrode at frequency 2 kHz, duty cycle 72  $\mu$ s, generator voltage 265 V, gap 4.5 cm, and the fastest gas flow rate (level 6, 0.16 m<sup>3</sup>/s). The negative peaks in Fig. 9 are caused by the plasma radiation itself (N<sub>2</sub> SPS). There is no emission when

Fig. 9 Typical UV–Vis absorption spectrum of the pulsed corona discharge, frequency 2 kHz, duty cycle 72  $\mu$ s, generator voltage 265 V, gap 4.5 cm, measured ~ 1 cm above the planar grounded electrode, the fastest gas flow rate (0.16 m<sup>3</sup>/s). Comparison of the measured spectrum with synthetic spectrum calculated for the absorption path 20 cm and O<sub>3</sub> concentration 106 ppm



the reference spectrum without  $O_3$  absorption is recorded (with plasma off), thus the  $N_2$  SPS emission appears as the negative absorption when plasma is on.

The exhaust suction speed and the associated gas flow rate influenced the ozone concentration significantly. The concentration of  $O_3$  increased as the suction weakened, but for safety reasons, to keep minimum O<sub>3</sub> concentration in laboratory, we mostly worked with the strongest suction speed (level 6, 0.16 m<sup>3</sup>/s). Under these conditions we measured the dependence of O<sub>3</sub> concentration on the discharge frequency and duty cycle. Figure 10 shows the dependence of  $O_3$  concentration on the duty cycle, but we can also see the increase of  $O_3$  concentration with the increasing frequency. The relative uncertainty of O<sub>3</sub> concentration shown in Fig. 10 is around 15% and it is mostly due to the fluctuation of  $O_3$  concentration inside the reactor. Based on the time variation (fluctuation) of the relative O<sub>3</sub> concentration calculated from the changes of the light intensity integrated over the spectral range 245-255 nm, we can assess that steady-state conditions were reached relatively quickly, within ~ 10 s (rise time of the  $O_3$  concentration from 10 to 90% of the mean steady-state concentration). This is in contrast to the data presented by Scally et al. [62] who used the same plasma source, but it was placed inside a closed box. They observed a slower increase of O<sub>3</sub> concentration after plasma was switched on, in the time scale of 100-200 s. Maybe it was due to much higher O3 concentrations observed in their experiments-above 10<sup>17</sup> cm<sup>-3</sup> (~5000 ppm). However, the relevance of their data is questionable, the claimed O3 concentration was too high, so it should be out of the range of their used UV-Vis absorption technique (saturated absorption).

#### SEM Analysis

The SEM analysis of the surfaces of pea seeds treated by plasma (direct and indirect PAW treatment) in comparison to the untreated ones are presented in the Tables 1 and 2. Regarding the direct treatment of pea seeds by pulsed corona discharge for 3 and 5 min, some morphological differences are observed as depicted in Table 1. At magnification 1000×, the control appeared with a relative uniform surface. In contrast the pea seeds exposed to plasma for 3 min and 5 min showed more cracks (represent by red arrows) on the seed





**Table 2** SEM images of pea seeds with seed control (C) and after indirect plasma treatment with PAW (TS 0.5, GD 1 and GD 2) at  $1000 \times$  and  $10,000 \times$  magnifications



surface. By increasing the magnification  $(10000\times)$ , the pineapple-eyes are well visible (red arrows) on the untreated pea seeds. After the plasma treatment, we observed a progressive



**Fig. 11** Effect of pulsed corona plasma priming of pea seeds on specific growth parameters after 8 days of growing. C is the control (the untreated seeds), PC-1, PC-3, PC-5 and PC-10 are pea seeds treated by pulsed corona plasma for 1, 3, 5 and 10 min, respectively. **a**, **b** and **c** represent the length, fresh weight (FW) and the dry weight (DW) of the shoot, respectively. **d**, **e** and **f** represent the length, fresh weight and the dry weight of the root, respectively. The values are the means of at least 3 independent experiments. The values are shown as mean  $\pm$  SD. Different letters indicate significant difference at *p* < 0.05, analyzed using ANOVA one test

disappearance of the pineapple-eye structure, which may indicate a physical plasma etching effect on the seed surface.

Table 2 showed the SEM of the surface of pea seeds imbibed in tap water (C) and PAW (TS 0.5, GD1 and GD 2) for 24 h, then dried. The results showed that at  $1000 \times$  magnification there was no significant difference except the seeds imbibed in GD 2, which showed a denser surface. This difference was confirmed at the magnification  $10,000 \times$  by the non-presence of the pineapple-eyes at its surface like in the control, TS 0.5 and GD 1. This difference could be related to the specific RONS (H<sub>2</sub>O<sub>2</sub>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>) in the generated PAW but further research is needed for more specific conclusions.

#### Effect of Direct Pulsed Corona Discharge on Pea Seedlings Growth

The results obtained from the preliminary germination and early growth tests of the pulsed corona plasma are depicted in Fig. 11. The tests were performed on the control (untreated seeds) and the seeds treated by pulsed corona plasma for 1, 3, 5 and 10 min designated as PC-1, PC-3, PC-5 and PC-10, respectively. The results show a significant improvement of germination and early growth for the seeds treated by pulsed corona discharge compared to control, especially for the shoots. We observed an improvement percentage of 20%, 23%, 47% and 31%, respectively, for PC-1, PC-3, PC-5, and PC-10 compared to control for the shoot length after 8 days of growing. However, the seeds treated for 10 min showed a decrease of shoot length compared to 5 min treatment but still significantly better compared to control, see Fig. 11a–c. The same trend is observed for the fresh and dry weight of the shoot, where the plasma treated seeds for 5 min seem to be the optimum treatment with an improvement percentage of 45% and 25%, respectively, for the fresh weight and dry weight.

Regarding the effect of the pulsed corona plasma on the pea roots after 8 days of growing, we also observed significant positive effects especially on root length and fresh weight, as shown in Fig. 11d-e. We obtained an improvement compared to control for the length and the fresh weight of the root of 23% and 34%, respectively. Unlike the seedlings shoot, we obtained the best results for the seedlings' root with the longest treatment time 10 min (PC-10). These positive results obtained for the shoot and root after 8 days of growing may be caused by the positive interaction of ozone and the UV radiation from the pulsed corona discharge and the surface of the pea seeds during the treatment. Several authors suggest that during the exposition of the seeds to the plasma source, the plasma induces some biochemical, chemical and physical changes in the treated seeds [63-68]. Since the pulsed corona discharge generated ozone up to 150 ppm, we compared our results with other works carried out using ozone. Sudhakar et al. [69], studied the effect of ozone concentration on tomato seed germination. They demonstrated that after 5 days of growing the seedlings exposed to ozone lead to the faster germination compared to control. They also showed that a high concentration of ozone and long treatment annihilated the germination compared to the control. Avdeeva et al. also documented some positive effect of seeds



**Fig. 12** Yield parameters evaluated after the harvest of the pea seeds primed with pulsed corona discharge for 3 (PC-3) and 5 min (PC-5), stored for 6 weeks, and soaked for 8 h in tap water before sowing, compared to the control (C). The graphs represent the average of: **a** pod weight per plant, **b** number of seeds per pod, **c** seed weight per group, **d** pods per plant, **e** total number of seeds per plant, **f** total seed weight per plant, **g** dry weight per plant. The values are shown as mean  $\pm$  SD

treated with ozone, they concluded that the germination depends on the concentration of ozone used and the higher ozone concentration seemed to be inhibiting the germination [70]. Normov et al. also reported the same effect, they concluded that by treating maize seed with ozone, the best germination was obtained for the lowest concentration [71]. These results suggest that the pulsed corona discharge used in this work and generating low ozone concentrations could be an efficient plasma source for pea seed priming. Bogdanov et al. stated that by using a concentration of 0.009 ppm for ozone to treat the zucchini seeds for a treatment time 247.7 min, the maximum germination rate is 79% [72].

#### Effect of Direct Pulsed Corona Plasma Priming on Pea Yield Parameters

The field experiments were conducted until harvest. We focused on the following pea yield parameters to evaluate the efficiency of the use of pulsed corona plasma as a priming source: pod weight, seeds per pod, seed weight, pods per plant, total seeds per plant, total seed weight, and dry weight of the plant. The yield parameters of the plasma treated seeds for 3 and 5 min (PC-3 and PC5) and the untreated seeds (C) are shown by the graphs in Fig. 12a-g. The effect of pulsed corona priming on pod weight per group is shown in Fig. 12a, which shows an improvement of weight by 11% and 19%, respectively for PC-3 and PC-5, compared to control. Figure 12b shows the results obtained after averaging the number of seeds per pod, we observed an improvement compared to control. Figure 12c shows the average seed weight per group: the seeds primed by pulsed corona have the higher average seed weight compared to the unprimed ones, with an improvement of 15% and 29% for PC-3 and PC-5, respectively. The other yield parameters, such as number of pods per plant, total number of seeds per plant and total seed weight per plant showed a significant enhancement for the primed seeds compared to the unprimed ones: for PC-3 39%, 78% and 105%, and for PC-5 29%, 79% and 131%, respectively, for the parameters pods per plant, total seeds per plant and total seed weight per plant. At the end of the plant growth, the dry matter was evaluated and showed that the biomass content from PC-3 was around 1.2-fold and from PC-5 about 1.6-fold higher compared to control.

These results showed that even after 6 weeks of storage post pulsed corona discharge priming, the positive effect on the seeds was still present. Similarly, Brust et al. [41] showed the long-term positive effect of the dielectric barrier discharge used to treat the winter barley and winter wheat seeds. They documented that even after one and two months of storage at room temperature and dry conditions, both seeds treated by plasma showed a germination higher than the control. They concluded that the dielectric barrier discharge used in their work with the specific characteristics and specific treatment times could be efficient to treat the winter barley and winter wheat, and the treated seeds can be stored for several weeks without losing the positive effect on germination. Tamosiune et al. [73] also reported that the storage of sunflower seeds treated by dielectric barrier discharge for one week did not affect the positive effect on the treated seeds. In this work ozone was found as the only reactive oxygen species. Not many studies have been carried out using ozone to treat seeds and the effect of storage. Nevertheless, Sudhakar et al. [69] showed that tomato seeds exposed to low concentration of ozone and stored for one and three months retained the improved germination efficiency compared to the non-treated ones.

Regarding the yield parameters, our very positive results can be explained by the fact that the exposure of the pea seeds to the pulsed corona plasma can be considered as physical priming. Factors like UV radiation, electric field, and reactive oxygen species,



**Fig.13** Effect of PAW seed priming on the average pod weight, point A-2020, point A-2021 and point B-2021. C for the control, TS 0.5 transient spark flow rate 0.5 ml/min, GD 1 and GD 2 for glow discharge treatment time 1 and 2 min, Nitric for the diluted nitric acid. The values are shown as mean  $\pm$  SD. Different letters (at point B) indicate significant difference at p < 0.05, analyzed using ANOVA one test

especially ozone influenced the pea seed coat and induced some stimulative response in the seeds. Waskow et al. [65] tried to explain that ozone in a presence of UV radiation from the plasma may generate superoxide and hydroxyl radicals and then interact with the seed coat. They suggested that this could be the critical stage to understand how the physical treatment of the seeds induces the internal biological changes. In the same way, Sudhakar et al. [69] stated that ozone interacts with the surface of the seed and then diffuses within the structure. They put forward that the reactive oxygen species could react with plant hormones, especially abscisic acid, which is important in the dormancy release and germination. Several authors reported some positive effects of the seeds treated by cold plasma on the yield, e.g. Roy et al. obtained a significant difference for the number of spikelet/spike and the number of grains/spike compared to control, for the wheat seeds treated by gliding arc discharge, especially for the treatment time of 6 min [74]. Li et al. [75] evaluated the effect on the yield of the treatment of peanut seeds by cold plasma at 120 W. They reported significant differences compared to control for the branch number per plant, pod number per plant, 100 pod weight, and yield per hectare. The positive effect on the yield using cold plasma for the treatment of seed was also reported by Jiang et al. [39] on wheat seeds, Zhou et al. [76] on green bean seeds, and Ivankov et al. [77] on buckwheat seeds.

#### Effect of Plasma Activated Water Pea Seed Priming on Yield Parameters

To investigate the feasibility of the yield improvement of pea, PAW was used as priming agent to carry out the tests in the outdoor field conditions. The plant yield parameters evaluated from the control and the PAW-primed seeds were the pods weight, seeds per pod, seed weight, pods per plant, total seeds and total seed weight per plant, and the dry weight per plant. Figure 13 shows the effect of PAW on the average pod weight per plant and per group. No improvement was found on the test carried out in 2021 in point A, while the experiment carried out in 2020 in point A and in 2021 at point B showed some positive effects. The improvement at the point A in 2020 was 25%, 21% and 10%, respectively, for TS, GD1 and GD 2; for point B in 2021 the improvement was 10%, 57%, 22% and 32%, respectively for TS, GD 1, GD 2 and dilute nitric acid. These results showed that the 24 h PAW-primed pea seeds, GD 1 seems to show the best effects.

Regarding the average number of seeds per pod, slight improvement was observed for the experiments carried out at the point A, while for the point B we obtained stronger 4%, 56%, 27% and 11% enhancement compared to control for TS, GD 1, GD 2 and Nitric, respectively, as shown in Fig. 14. These results may be influenced by the soil

composition, which may play an important role in the effect during the growing process, depending on if the soil was with compost (point A) or a non-enriched sandy (point B).

We also investigated whether the PAW-priming of seeds affected the average seed weight of the different groups after the harvest. The result is depicted in Fig. 15. We observed no effect on the seed weight at point B except a slight increase of 10% for the seeds primed with diluted nitric acid compared to control. At point A, we observed an improvement in 2020 of 7% and 13%, respectively, for TS and GD 1, and in 2021 an improvement of 1%, 3% and 12%, respectively, for TS, GD 2 and Nitric.



Fig. 14 Average seed per pod for the pea seeds primed with PAW, point A-2020, point A-2021 and point B-2021. C for the control, TS 0.5 transient spark flow rate 0.5 ml/min, GD 1 and GD 2 for glow discharge treatment time 1 and 2 min, Nitric for the diluted nitric acid. The values are shown as mean  $\pm$  SD. Different letters (at point B) indicate significant difference at p < 0.05, analyzed using ANOVA one test



**Fig. 15** Average seed weight for the pea seeds primed with PAW, point A-2020, point A-2021 and point B-2021. C for the control TS 0.5 transient spark flow rate 0.5 ml/min, GD 1 and GD 2 for glow discharge treatment time 1 and 2 min, Nitric for the diluted nitric acid. The values are shown as mean  $\pm$  SD. Different letters (at point B) indicate significant difference at p < 0.05, analyzed using ANOVA one test



**Fig. 16** Average pod per plant for the pea seeds primed with PAW, point A-2020, point A-2021 and point B-2021. C for the control TS 0.5 transient spark discharge flow rate 0.5 ml/min, GD 1 and GD 2 for glow discharge treatment time 1 and 2 min, Nitric for the diluted nitric acid. The values are shown as mean  $\pm$  SD. Different letters (at point B) indicate significant difference at p < 0.05, analyzed using ANOVA one test



**Fig. 17** Total seeds per plant for the pea seeds primed with PAW, point A-2020, point A-2021 and point B-2021. C for the control TS 0.5 transient spark flow rate 0.5 ml/min, GD 1 and GD 2 for glow discharge treatment time 1 and 2 min, Nitric for the diluted nitric acid. The values are shown as mean  $\pm$  SD. Different letters (at point B) indicate significant difference at p < 0.05, analyzed using ANOVA one test



**Fig. 18** Total seed weight per plant for the pea seeds primed with PAW, point A-2020, point A-2021 and point B-2021. C for the control TS 0.5 transient spark flow rate 0.5 ml/min, GD 1 and GD 2 for glow discharge treatment time 1 and 2 min, Nitric for the diluted nitric acid. The values are shown as mean  $\pm$  SD. Different letters (at point B) indicate significant difference at p < 0.05, analyzed using ANOVA one test

The improvement of the number of pods per plant was observed on the tests carried out at both fields. As shown in Fig. 16, for point B we obtained an improvement of 1.33-fold, 1.35-fold, 1.58-fold and 1.50-fold, respectively, for TS, GD 1, GD 2 and Nitric compared to control. Similar improvement was also observed at point A in 2021 with 1.39-fold, 1.43-fold, 1.32-fold and 1.19-fold improvement compared to control, respectively, for TS, GD 1, GD 2 and Nitric.

The effect of the PAW seed priming on the total seeds per plant was also evaluated. As shown in Fig. 17, the pea seeds primed with PAW showed an improvement for both fields. For the tests performed at point B, we obtained an enhancement of 36%, 106%, 98% and 61% compared to control, for point A in 2021, the enhancement was 36%, 48%, 10% and 15% compared to control for TS, GD 1, GD 2 and Nitric, respectively. Plants grown from seeds primed for 24 h by GD 1 PAW showed a higher number of seeds per plant compared to control and other PAW primed seed groups. Nevertheless, all the groups of seeds primed by both PAWs and nitric acid showed a better yield in the total number of seed per plants. At the end we evaluated the total seed weight per plant, as shown in Fig. 18. PAW helped to improve the total seeds per plant for both points A and B in comparaison to the control.

Since no previous work was found in the literature on the use of PAW as a priming agent to improve the yield of harvested seeds, we measured the long-life species present in PAW (hydrogen peroxide, nitrate and nitrite ions) to assess their effects on possible phenomena occurred during the seed priming, which induced the enhancement of the yield.

These positive effects on yield parameters may be associated to two main factors: the coat modification and the adsorption of the active species by the seeds during the 24 h priming. Regarding the seed surface, Rathore et al. and Sajib et al. [35, 78] showed that when soaking pea seeds and black green seeds for 24 h, the coats of the treated seeds are modified; the coat morphology was evaluated by scanning electron microscopy. They assumed that the morphology change could be attributed to the reactive oxygen and nitrogen species present in the PAW during the 24 h soaking. Also, these reactive species removed the protecting natural wax from the seed surface during the interaction and helped the PAW-primed seeds to become more hydrophilic. On the other hand, these results could be associated with the interaction of the pea seeds with the PAW through the uptake of the reactive oxygen and nitrogen species from PAW during the 24 h priming. Kučerová et al. [79] studied the interaction of the wheat seeds and long-life species from PAW generated by transient spark. They soaked the seeds in the fresh produced PAW and evaluated the concentration of hydrogen peroxide, nitrate and nitrites ions for several hours and days after soaking the seeds. Their results showed that the concentration of hydrogen peroxide, nitrate and nitrites ions, which were in contact with seeds decreased faster than in the samples without seeds. These reactive species adsorbed through the priming then interact with the enzymes in the seeds and induce the physiological, molecular and biochemical changes [5, 19], which could have led to the increase of the yield for the seeds primed with PAW.

# Conclusion

Plasma agriculture is an emerging field applying new advances of plasma science into agriculture. A lot of research has been carried out mostly at the laboratory scale and have shown promising results of improved seed germination and enhanced plant growth upon cold plasma treatment. The application potential of these results needs to be validated by translating them into the field. This work contributes to plasma agriculture with a novel approach of the use of cold plasma both in direct and indirect (plasma activated water) way for pea seed priming. We evaluated various plant growth parameters of the harvested pea plants grown from the primed seed and focused to the yield improvement. The results obtained throughout this work, can be concluded as follows:

- After 6 weeks of storage, the pea seeds treated directly by pulsed corona discharge showed better yield parameters compared to the untreated seeds.
- The key reactive oxygen species of the pulsed corona plasma is ozone, reaching concentrations up to 150 ppm. The pea plants grown from seeds primed the by pulsed corona discharge and stored for 6 weeks before sowing in the outdoor field, showed substantially improved the pea yield parameters for both 3 and 5 min treatment times.
- The pea plants grown from pea seeds primed in PAW for 24 h showed slight improvements compared to control, especially in the number of seeds per pod and the total number and weight of seeds.
- The SEM analysis carried out on the pea seeds from direct plasma and GD 2 indirect treatments revealed some surface morphology differences (cracks and surface pattern change) compared to the untreated control, which indicates that plasma treatment has direct physical effect on the seed surface in addition to the chemical effects of RONS.

- The long-lived reactive species (hydrogen peroxide, nitrate and nitrites) in PAW are most likely responsible for the improvement of the yield obtained after the indirect plasma priming of the pea seeds.
- Both direct or indirect cold plasma treatments demonstrated a potential to improve the agricultural plant yield and so may lead to the reduced use of fertilizers.

These results support the vision that the emerging field of plasma agriculture has a great application potential. It should be devoted a high interest because it may represent the way to alleviate negative environmental effects of the use chemical fertilizers in agriculture, while increasing the product yield.

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# Declarations

Conflicts of interest The authors declare no conflict of interest.

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