Heliyon 10 (2024) e38160

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Impact of dielectric barrier discharge plasma and plasma-activated water on cotton seed germination and seedling growth

Mohammad Hossein Mohajer [a], Motahare Monfaredi [a], Maede Rahmani [a], Mahdye Martami [a], Elahe Razaghiha [a], Mohammad Hossein Mirjalili [b], Aidin Hamidi [c], Hamid Reza Ghomi [a,*]

[a] Shahid Beheshti University, Laser and Plasma Research Institute, Shahid Beheshti University, 1983969411, Tehran, Iran
[b] Department of Agriculture, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, 1983969411, Tehran, Iran
[c] Agriculture Research, Education and Extension Organization (AREEO), Seed and Plant Certification and Registration Institute (SPCRI), Karaj, Iran

ARTICLE INFO

Keywords:
Agricultural science
Non-thermal plasma
Surface treatment
DBD
PAW
Electrical conductivity

ABSTRACT

Unfavorable environmental conditions during planting can reduce seed germination and hinder seedling growth. To address this issue, manufacturers are exploring innovative and cost-effective methods, such as cold plasma discharge. This simple, low-cost, and efficient physical technique induces significant biological responses in seeds and plants without the use of traditional, environmentally hazardous chemicals. This study investigated the impact of dielectric barrier discharge (DBD) plasma and plasma-activated water (PAW), produced by gliding arc plasma, on the germination and seedling growth of My344 cotton seeds. The seeds were pre-treated with 80 W of DBD plasma for 0, 1, 2, and 3 min, and subsequently soaked for 90 min in PAW with varying pH levels of 5.82, 3.88, 3.63, and 3.38. The results showed that plasma treatment positively influenced seed germination and seedling growth. The highest germination percentage (98.89 %) was observed with 1 min of DBD treatment, followed by PAW priming at pH levels of 3.63 and 3.38. Additionally, a 3-min DBD treatment followed by soaking in PAW with a pH of 3.63 led to significant increases in stem length (76.76 %), root length (48.77 %), and wet weight (76.44 %). Furthermore, it was observed that the electrical conductivity of the seeds in all groups decreased significantly with increased PAW acidity. The physical and chemical effects of cold DBD plasma on the seed surface, as well as changes in hydrophilicity, were further examined using scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and water contact angle imaging.

1. Introduction

In agriculture, the successful cultivation of crops relies heavily on the effective germination, emergence, and early growth stages of planted seeds. These stages are crucial for meeting the increasing demand for food and materials such as cotton. For instance, in cotton farming, poor seed sprouting and weak early plant growth can significantly reduce cotton yield. Additionally, the establishment of a robust crop depends not only on seed quality and planting practices but also on various environmental factors such as temperature fluctuations, water scarcity, and soil salinity, which can pose significant challenges to successful crop establishment. Effectively

* Corresponding author.
E-mail address: h-gmdashty@sbu.ac.ir (H.R. Ghomi).

https://doi.org/10.1016/j.heliyon.2024.e38160
Received 29 May 2024; Received in revised form 14 September 2024; Accepted 18 September 2024
Available online 19 September 2024

addressing these factors is essential for meeting the ever-growing demands of modern agriculture [1]. Currently, several methods are employed to address germination issues, including the use of chemical sanitizers, organic and inorganic acids, hormones, scarification, and genetic modification of seeds. However, some of these methods have issues such as toxicity, damage, or high costs. Therefore, there is a pressing need in agriculture for an alternative solution that is both cost-effective and environmentally safe. This solution should thoroughly sanitize seeds without the risks or expenses associated with current methods, underscoring the importance of continued research and innovation for improved crop management and yield [2–4].

Nowadays, NTP (non-thermal plasma) technology has emerged as a promising innovation in the agricultural and food packaging sectors, offering an alternative or complementary approach to enhancing plant growth and reducing pathogen and chemical contamination in seeds. NTP refers to an ionized gas generated through various methods, such as microwaves, radio frequency, pulsed or alternating currents, in different gas mixtures and setups, including dielectric barrier discharge (DBD), atmospheric pressure plasma jet (APPJ), and corona discharges. These plasma systems are characterized by a unique feature: while heavy particles like ions and molecules remain at low temperatures, making them suitable for treating delicate material surfaces, the high electron temperature leads to the production of reactive oxygen and nitrogen species (ROS and RNS) and UV radiation, which effectively contribute to the decontamination and sterilization of surfaces exposed to NTP discharges [5].

In agriculture, researchers have harnessed these effects of plasma technology to deliberately induce stress, thereby selectively promoting seed germination [6,7], decontaminating seed surfaces [8], enhancing seed disease resistance [9], and fostering biochemical processes linked to increased crop yields [10]. A notable advantage of plasma treatment is its ability to simultaneously decontaminate seed surfaces and promote germination and growth using the same dosage. This dual functionality is particularly valuable as it ensures the safety and quality of seeds as a food source without compromising their integrity, as highlighted by previous studies [6].

In addition to the etching-related increase in the imbibition rate, the application of air-derived cold plasma further enhances seed germination rates through surface functionalization and the deposition of bioactive compounds [11]. This surface modification induced by air plasma alters both the physical and chemical properties of the seed coat, which effectively breaks dormancy and initiates germination [12]. The plasma environment generates bioactive molecules that adhere to the seed surface and penetrate up to 10 nm into the seed [13], acting as a stimulatory coating for germination. These molecules serve as a stimulatory coating for germination. Since atmospheric air is primarily composed of nitrogen and oxygen, air plasma discharge produces molecular nitrogen and oxygen, atomic nitrogen, and atomic oxygen. Under highly reactive plasma conditions, nitrogen oxide molecules such as NO, NO2, and N2O are also produced [14,15]. These species are physiologically significant in biological systems and may directly promote germination processes while selectively disinfecting surface-dwelling pathogenic fungi and bacteria without harming the cotyledon [16,17].

Furthermore, plasma-activated water (PAW) has demonstrated its efficacy in promoting seed and seedling germination, fostering plant development, and combating plant-related pathogenic bacteria and fungi-infected seedlings [18–21].

Research into the effects of cold plasma treatment on water has highlighted its ability to eliminate organic contaminants and promote the production of reactive species such as H2O2, NO3−, and NO2−. Plasma-activated water is derived from various sources such as tap water, distilled water, or demineralized water, which are exposed to plasma-generating electrical discharge in different environments, including vacuum, air, inert gases, or others, using various reactor configurations [22]. Studies have shown the positive impact of PAW on germination rates and plant growth [5,23–25].

These beneficial effects of PAW in agricultural contexts are attributed to the synergistic actions of various reactive species, particularly oxygen and nitrogen species, generated during the plasma treatment of water [26–28]. These reactive species are believed to participate in plant signaling pathways, regulating metabolic processes, facilitating plant development, and enhancing responses to diverse stresses. This cascade of effects ultimately contributes to improved germination rates and enhanced plant growth, underscoring the potential of PAW as a sustainable solution in agriculture [27,29,30].

2. Materials and methods

This research was conducted at the Advanced Laser-Plasma Research Institute and the Medicinal Plants Research Institute of Shahid Beheshti University in 2023. Cotton seed samples of the certified foreign variety My344 were obtained from the Iran Seed and Sapling Registration and Certification Institute for this study. The seeds were pre-treated with cold atmospheric plasma using a DBD setup for durations of zero (D0), one (D1), two (D2), and three (D3) minutes at 80 W [3]. Subsequently, the seeds were soaked for 90 min in water treated by gliding arc plasma to produce plasma-activated water (PAW) with varying pH levels. Distilled water was treated for zero (P0 = pH 5.82), two (P1 = pH 3.88), four (P2 = pH 3.63), and six (P4 = pH 3.38) minutes. The experiment was conducted using a factorial 4 × 4 design, which involved four durations of direct seed treatment with DBD and four durations of seed priming with PAW, all within a completely randomized design with three replications, each consisting of 30 seeds.

The electrical conductivity (EC) of seed leachates was measured to assess the extent of electrolyte leakage from plant tissues, which provides an estimate of seed vigor. EC rates in seeds were recorded before and after PAW treatment to determine the changes in seed EC. Treated seeds were placed in sterilized containers between two filter papers and incubated at 25 °C. After checking the percentage and speed of germination, the seeds were transferred to culture trays after 3 days and kept for 10 days. Then, 10 seedlings were randomly selected from each culture to measure physical characteristics.

The general characteristics measured included germination percent (GP), germination speed (GR), stem length (SL), root length (RL), fresh weight (FW), dry weight (DW), and EC. For the samples treated with the optimal voltage and the control sample, additional tests were conducted to evaluate the physicochemical modifications induced by Dielectric Barrier Discharge (DBD) plasma on seed surfaces, including Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR) [31–33], and water droplet contact angle measurements. The optical emission spectrometry of DBD and gliding arc plasmas was measured to determine the species present in the plasma.

The shoot and root lengths of seedlings were measured using a ruler, while seedling fresh and dry weights were recorded using a digital scale. Other indices are presented in Table 1.

For the direct treatment of seeds in this study, the DBD Enhancedtech-151 device was used, and PAW was generated using the Plasmatek-15B plasma generator manufactured by Kaavosh Yaran Fan Puya Company (see Figs. 1 and 2). DBD plasma was generated using pulsed direct current voltage with a frequency of 6 kHz and a variable output of 10–24 kV for the upper electrode, and a frequency of 50 Hz with an output of 0–24 kV for the lower electrode. Samples were placed in Pyrex dishes (dielectric, diameter 22 cm, height 10 cm, thickness 4 mm) between the two electrodes. The experiment was conducted at atmospheric pressure, with treatments lasting 1, 2, and 3 min at 80 W (24 kV).

For PAW, the distance from the gliding arc setup nozzle tip to the water surface was 2 cm. Distilled water with a pH of 5.82 was used for the experiment, and the output power of the gliding arc device was 2100 W.

3. Results

The data analysis revealed that PAW treatment had a significant impact ($p < 0.05$) on all the parameters examined, except for the germination percentage. Similarly, DBD treatment significantly influenced ($p < 0.05$) all parameters except for the germination rate. When PAW treatment was combined with DBD treatment, there was no significant change in germination percentage, consistent with the effect observed with PAW alone. The subsequent sections will describe the measurements of seed and seedling parameters.

3.1. Seed germination percentage

Statistical analysis at a 95 % confidence interval showed that DBD treatment was an effective factor in influencing germination percentage, while PAW treatment and the interaction between PAW and DBD did not significantly affect cotton seed germination. The highest germination percentage (98.89 %) was observed with a 1-min DBD treatment followed by PAW priming at pH 3.63 and 3.38 (D1P2 and D1P3). The germination percentage then decreased linearly with increasing DBD treatment duration. For priming with non-activated water, the highest germination was observed with 1 and 3 min of DBD treatment (D1 and D3), both achieving 96.67 %. The lowest germination rate (85.55 %) was recorded in the untreated control (D0P0). The effects of different DBD treatment times and PAW treatments are illustrated in Fig. 3.

3.2. Germination rate

The study also examined the impact of PAW priming and DBD treatment on the germination rate (speed) of seeds (Fig. 4). Statistical analysis revealed that PAW priming and the interaction between PAW and DBD were significant factors influencing germination rate ($p < 0.05$), whereas DBD treatment alone was not. The highest germination rate (14.97 seeds/day) was observed with the combined treatment of 1-min DBD and PAW at pH 3.38 (D1P3). The untreated control seeds (D0P0) had the lowest germination rate of 12.7 seeds/day.

3.3. Stem length and root length of the seedlings

All DBD and PAW treatments significantly affected stem length ($p < 0.005$) (Fig. 5). The best result was obtained with a 3-min DBD treatment followed by PAW priming at pH 3.63 (D3P2), which resulted in a 76.76 % increase in stem length compared to the control group. The worst result was observed with a 3-min DBD treatment alone (D3P0), where stem length decreased by 41.21 % compared to the control group.

All treatments also significantly affected root length ($p < 0.05$) (Fig. 6). The best result was obtained with a 3-min DBD treatment followed by PAW priming at pH 3.63 (D3P2), leading to a 23.77 % increase in root length compared to the control group. The worst result was observed with a 1-min DBD treatment alone (D1P0), where root length decreased by 48.77 % compared to the control group.

Table 1
Total number of seeds and Ni: Germinating seeds at the end of 3rd day, ni: germinated seeds per day, and di: counting day.

Studied indices	Calculation formula	Unit of measurement
Germination percent [34]	$GP = \frac{N_i}{N}*100$	%
Germination rate [34]	$GR = \sum \frac{n_i}{d_i}*100$	Number/day
Root length	ruler	*Cm*
Stem length	ruler	*Cm*
Fresh weight	Laboratory digital scale	g
Dry weight	Laboratory digital scale	g
EC [35]	EC = EC sample - EC water	$\mu S\ cm^{-1} g^{-1}$

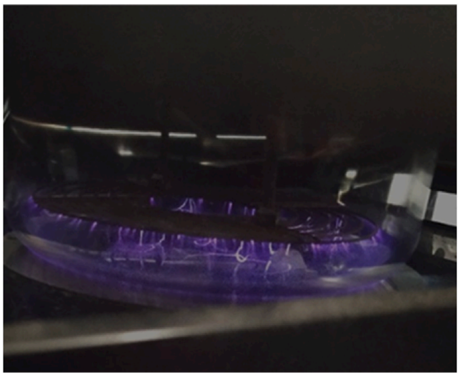

Fig. 1. Plasma produced by DBD Enhancedtech-151.

Fig. 2. Expusuring distild water to gliding arc plasma produced by Plasmatek-15B

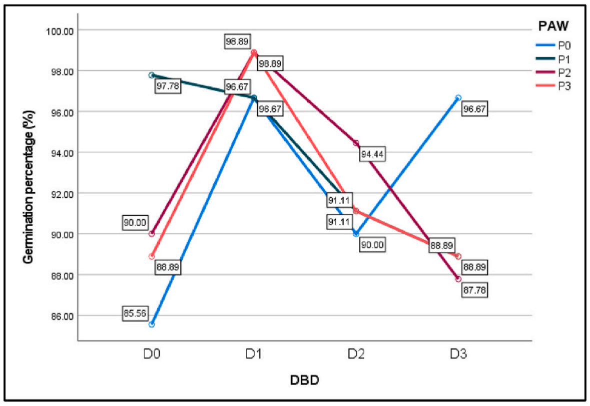

Fig. 3. This graph shows the changes in the germination percentage of cotton seeds. Different colors represent different pH values (P0 = 5.82, P1 = 3.88, P2 = 3.63, and P3 = 3.38) combined with different DBD time (D0 = 0min, D1 = 1min, D2 = 2min, D3 = 3min). The highest germination percentage (98.89 %) was observed in D1P2 and D1P3.

The data demonstrated that the combination of DBD treatment and PAW priming enhanced both stem and root lengths compared to controls with D3P2 yielding the best results. However, DBD treatment alone (P0) could negatively impact growth depending on the duration, indicating that longer treatment times are necessary to optimize the effects.

3.4. Wet and dry weight of the seedlings

The data showed that all treatments significantly impacted fresh plant weight ($p < 0.05$). The most effective treatment was a combination of 3 min of dielectric barrier discharge (DBD) followed by 3.63 pH of plasma-activated water (PAW) priming (D3P2),

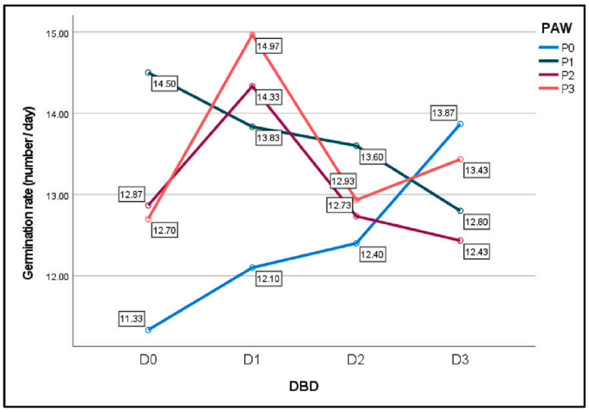

Fig. 4. This graph shows the changes in the germination rate of cotton seeds. Different colors represent different pH values (P0 = 5.82, P1 = 3.88, P2 = 3.63, and P3 = 3.38) combined with different DBD time (D0 = 0min, D1 = 1min, D2 = 2min, D3 = 3min). The highest germination rate (14.97 number/day) was observed in D1P3. The untreated control seeds (D0P0) had the lowest germination rate of 12.7 seeds per day.

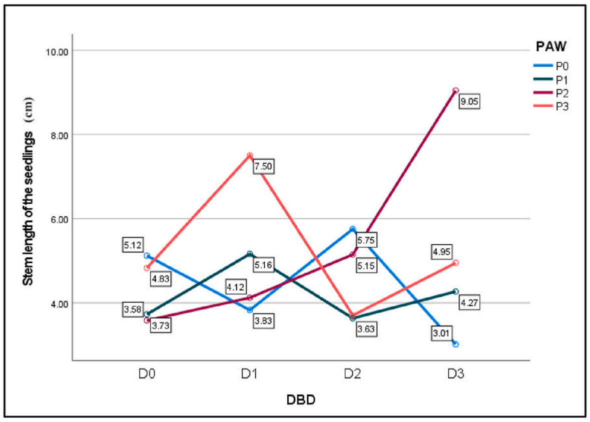

Fig. 5. This graph depicts the stem length of seedlings. Different colors represent different pH values (P0 = 5.82, P1 = 3.88, P2 = 3.63, and P3 = 3.38) combined with different DBD time (D0 = 0min, D1 = 1min, D2 = 2min, D3 = 3min). The best result was obtained in D3P2 which increased by 76.76 % compared to the control group.

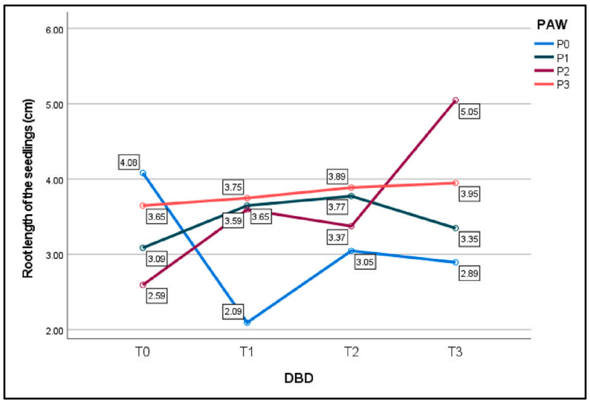

Fig. 6. This graph depicts the root length of seedlings. Different colors represent different pH values (P0 = 5.82, P1 = 3.88, P2 = 3.63, and P3 = 3.38) combined with different DBD time (D0 = 0min, D1 = 1min, D2 = 2min, D3 = 3min). The best result was obtained in D3P2 which increased by 23.77 % compared to the control group.

which increased fresh weight by 76.44 % compared to the control group. In contrast, the least effective treatment was 3 min of DBD alone (D3P0), which decreased fresh weight by 28.32 % compared to the control. For PAW primings of P0 = 5.82, P1 = 3.88, and P3 = 3.38, the fresh weight exhibited a sinusoidal trend concerning DBD treatment time, while P2 = 3.63 showed an upward trend (Fig. 7).

Additionally, all treatments significantly impacted seedling dry weight. The most effective treatment for increasing dry weight was 2 min of DBD followed by 3.88 pH of PAW (D2P1), which increased dry weight by 53.44 % compared to the control. Conversely, the least effective treatment was 3 min of DBD alone (D3P0), which decreased dry weight by 13.8 % compared to the control. For PAW primings of P1 = 3.88, P2 = 3.63, and P3 = 3.38, dry weight exhibited a sinusoidal trend concerning DBD treatment time, while P0 = 5.82 showed a downward trend (Fig. 8).

3.5. Electrical conductivity test (EC)

In this study, the electrical conductivity (EC) test was conducted to evaluate the effect of plasma treatment on seed germination. This rapid and reliable test assesses the condition of seed cellular membranes, which play a crucial role in germination, by measuring the amount of leachates and electrolytes released into the soaking solution. The EC test can determine whether plasma treatment improved or degraded the integrity of cellular membranes, consequently impacting germination potential [30,32,34]. Based on Fig. 9, the highest amount of leachate was released when seeds were untreated. In the PAW treatment groups, minimal leakage of cell electrolyte materials was observed, particularly with pH P3 = 3.38, followed by P2 = 3.63 and P1 = 3.88, with a notable difference from the control (about 60 %). When combining DBD with PAW treatment, it was inferred that increasing DBD treatment time from 1 to 3 min initially increased EC but then reduced it. In treatments with only DBD, a decreasing trend was observed as DBD time duration increased. Overall, PAW itself had a significant effect on reducing seed leakage.

3.6. Scanning electron microscope (SEM)

The effects of atmospheric air plasma treatment on cotton seeds were examined, specifically focusing on the influence of exposure time on seed surface topography. Conducted over durations of 1, 2, and 3 min, the atmospheric air plasma treatment induced notable changes in the seed's surface morphology, as observed through scanning electron microscope (SEM) imaging (Fig. 10). It was observed that prolonged plasma exposure led to increased surface damage. These alterations are hypothesized to facilitate water uptake and imbibition by creating microchannels within the seed coat.

3.7. Fourier transform Infrared Spectroscopy (FTIR)

Untreated and plasma-treated cotton seeds (those treated by DBD) were analyzed using Fourier Transform Infrared Spectroscopy (FTIR) to identify the functional groups and bonds present on the seed coat. The FTIR spectra in the range of 600–4000 cm^{-1} revealed the functional groups on the seed surface (Fig. 11). The increase in the intensity of the hydroxyl group (O-H) peak at 3300 cm^{-1} in the FTIR spectrum of plasma-treated seeds can be attributed to the interaction between the seed surface and reactive oxygen species (ROS) present in the DBD plasma. During plasma treatment, various reactive species are generated, including oxygen radicals (O•), hydroxyl radicals (OH•), and ozone (O_3). These reactive oxygen species can interact with the seed surface in several ways.

1. **Direct oxidation:** Oxygen radicals can directly oxidize surface molecules, creating new hydroxyl groups.

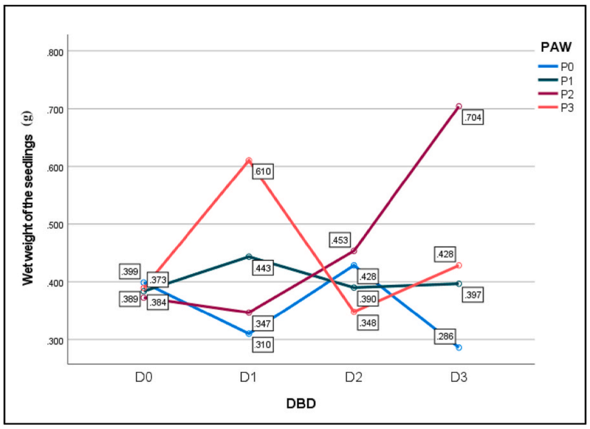

Fig. 7. This graph shows the wet weight of seedlings. Different colors represent different pH values (P0 = 5.82, P1 = 3.88, P2 = 3.63, and P3 = 3.38) combined with different DBD time (D0 = 0min, D1 = 1min, D2 = 2min, D3 = 3min). The most effective treatment was D3P2 which increased the fresh weight by 76.44 % compared to the control group.

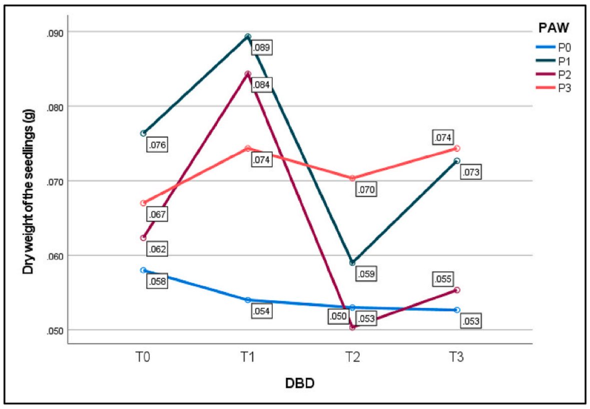

Fig. 8. This graph shows the dry weight of seedlings. Different colors represent different pH values (P0 = 5.82, P1 = 3.88, P2 = 3.63, and P3 = 3.38) combined with different DBD time (D0 = 0min, D1 = 1min, D2 = 2min, D3 = 3min). The most effective treatment for increasing dry weight was D2P1 which increased the dry weight by 53.44 % compared to the control.

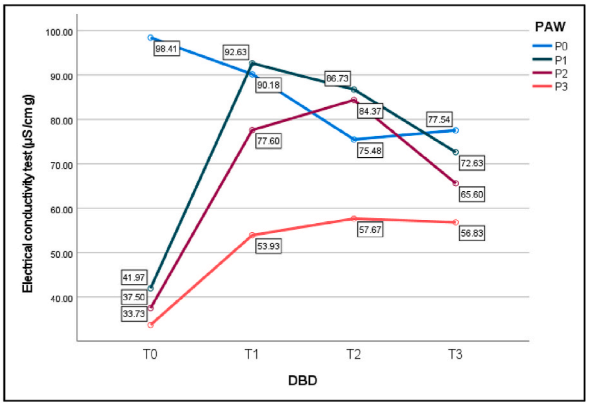

Fig. 9. This graph shows the change in EC of seeds. Different colors represent different pH values (P0 = 5.82, P1 = 3.88, P2 = 3.63, and P3 = 3.38) combined with different DBD time (D0 = 0min, D1 = 1min, D2 = 2min, D3 = 3min). PAW showed a significant impact on EC and with a pH of 3.38 it reduced by about 60 %.

2. **Hydrogen abstraction:** Hydroxyl radicals can abstract hydrogen atoms from surface molecules, leaving behind sites for hydroxyl group formation.
3. **Chain scission:** Plasma species can break polymer chains in the seed coat (e.g., cellulose, lignin), exposing more sites for hydroxylation.

The increase in hydroxyl groups on the seed surface is directly related to plasma species because these reactive species are responsible for the chemical modifications observed. This increase in hydroxyl groups contributes to enhanced surface hydrophilicity, as hydroxyl groups are polar and can form hydrogen bonds with water molecules. Furthermore, the observed increases in peaks related to C-N, N-H, and C=O groups (1400, 1550–1560, 1735, 1743 cm^{-1}) also support the interaction of plasma species (including nitrogen-containing species) with the seed surface, resulting in the incorporation of various polar functional groups [36–38].

3.8. *Measuring water contact angle*

Water contact angle measurements are essential in evaluating plasma treatment effects on seed surfaces, providing crucial insights into seed coat hydrophilicity/hydrophobicity. This non-destructive technique quantifies surface changes, indicates surface activation, and aids in optimizing treatment parameters. Decreasing contact angles post-treatment signifies successful surface modification and incorporation of hydrophilic functional groups, potentially correlating with improved water uptake and germination rates [39]. Previous studies on plasma activation of seeds showed that effective plasma etching can affect seed hydrophilicity and may improve water permeation into the endosperm and embryo. Whether these functional groups may act as reactive oxygen and nitrogen species (RONS) or include peroxide-like species known for deactivating the germination-retardant hormone Abscisic acid (ABA) is debated [40]. The plasma treatments effectively transformed the cotton seed surface from hydrophobic to hydrophilic by etching. Water

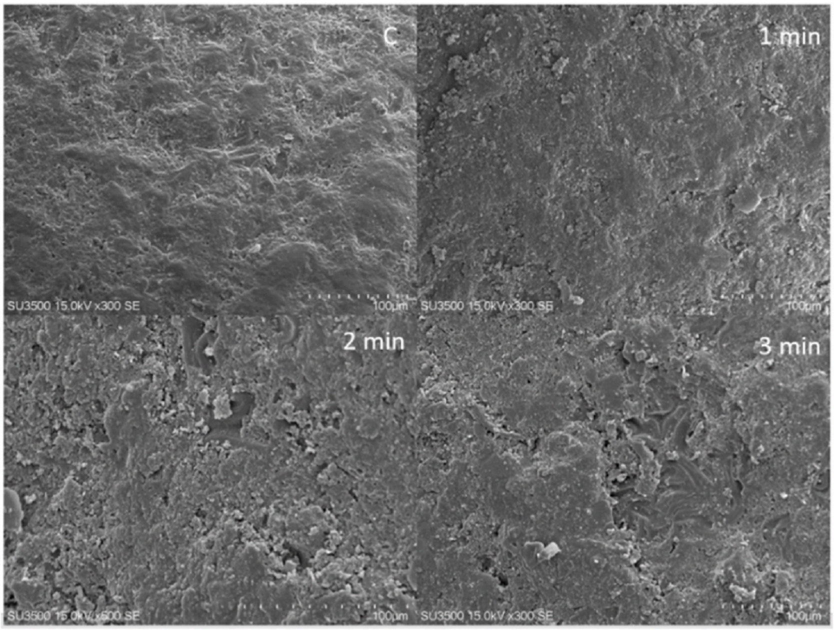

Fig. 10. Impact of DBD atmospheric plasma on cotton seed coat. C: untreated surfaces. Other photos show the effect of 1,2 and 3 min of treatment. As it is shown, with the increase in the treatment time, the surface destruction has increased.

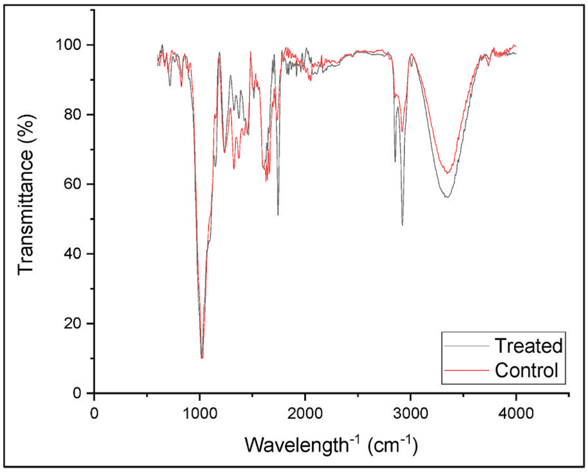

Fig. 11. Fourier Transform Infrared Spectroscopy (FT-IR) shows increasing in some areas related to C-N, N-H (1400, 1550–1560,1735 cm^{-1}), O-H (3300 cm^{-1}), and C=O (1735 cm^{-1} and 1743 cm^{-1}) groups.

droplets spread more on treated than untreated seeds (Fig. 12). With increasing exposure time, the droplet contact angle on the seed surface decreased. Water contact angle measurements were performed using a custom-built setup at the Laser and Plasma Research Institute of Shahid Beheshti University. The setup consists of a camera and, opposite to it, a seed holder and a water syringe holder. A water droplet is gently placed on the seed using the syringe, and the image is captured immediately (Fig. 13).

3.9. Optical emission spectroscopy (OES)

Subsequently, the optical emission spectroscopy (OES) of two plasma regimes used in this experiment to identify the chemical species present in the plasma was investigated. The spectrometer utilized in this experiment was an Avantes model. For spectral acquisition from the plasma generated by the Enhancedtech-15I (DBD) device, a window on the plasma chamber was used, the spectrometer's optical fiber was securely positioned facing this window, and the plasma spectrum was recorded (Fig. 14-A). For spectral acquisition from the Plasmatech-15B device (Gliding arc), the probe was placed at a safe distance from the plasma torch flame, and its spectrum was recorded too (Fig. 14-B). Using the NIST database and reviewing similar studies, the species corresponding to

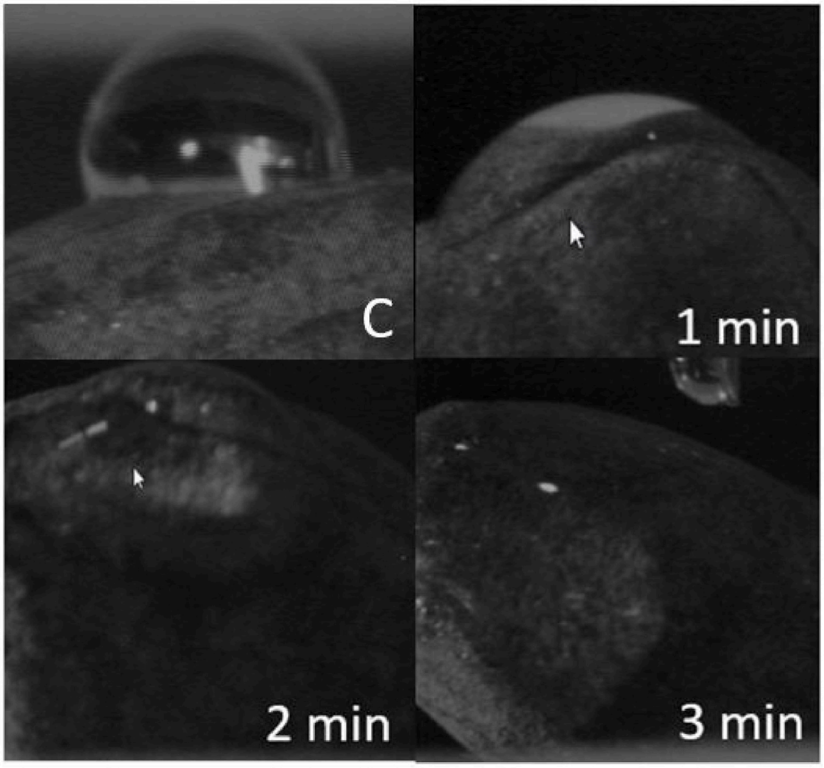

Fig. 12. C is the control seed. The other photos are related to DBD plasma treatment for 1, 2, and 3 min. As it is observed, with the increase of time, the hydrophilicity of the seed surface increases and the contact angle of the droplet decreases. In the 3 min of treatment, the droplet is completely spread over the surface of the seed.

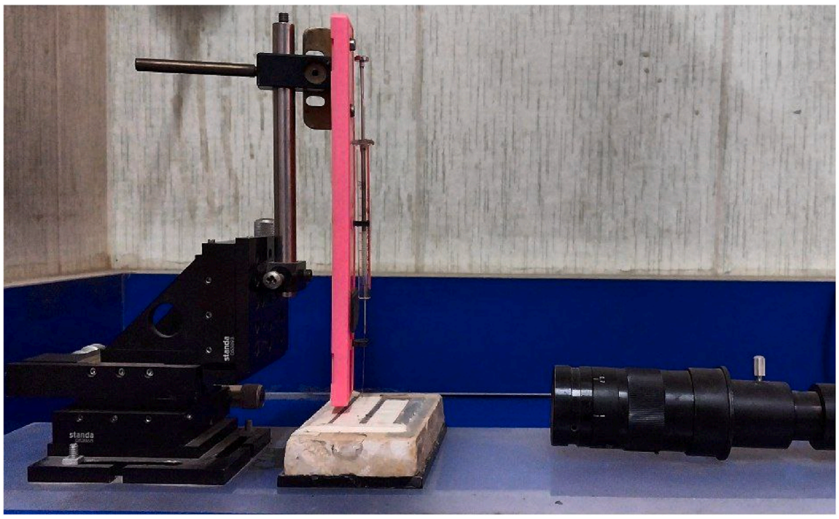

Fig. 13. Schematic of water droplet contact angle measurement device.

each peak were identified. Both plasmas contain various reactive oxygen and nitrogen species. As can be observed, most of the peaks are related to active nitrogen species [41].

4. Discussion

As demonstrated in Fig. 3, the highest germination percentage (98.89 %) was seen in the 1-min DBD treatment followed by a pH of

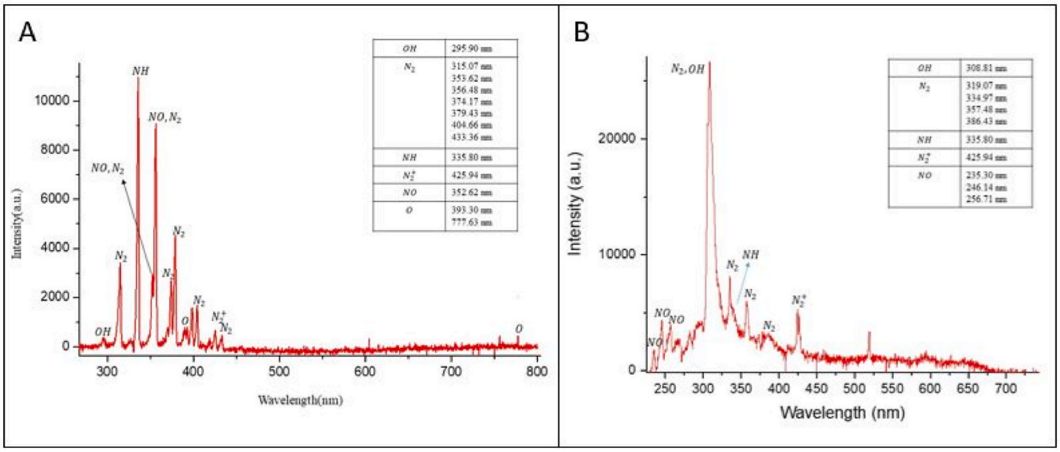

Fig. 14. A The optical emission spectroscopy of DBD plasma, B The optical emission spectroscopy of gliding arc plasma are shown at atmospheric pressure. The highest peak intensity is related to active nitrogen and oxygen species.

3.63 and 3.38 PAW priming (D1P2 and D1P3). Even in the distilled water priming treatment, the 1 and 3-minute DBD treatments (P0D1, P0D3) achieved germination percentages of 96.67 % and 96.66 %, respectively. Compared to the 80 % minimum national germination standard [42] direct cold plasma treatment increased germination by over 15 %. Across all DBD treatments combined with PAW, the direct 1-min DBD treatment (D1) consistently resulted in the highest germination rates (32.13 % increase compared to the control group). Based on Fig. 4, it is observed that increasing the time in the treatment of only DBD plasma increases the germination rate, but increasing the time in the combination of DBD plasma and PAW priming has an almost downward effect on the seed germination rate. Notably, the control treatment without any DBD plasma or PAW priming (D0P0) exhibited the lowest germination rate of 11.33.

These findings align with numerous studies [43–47] that have demonstrated the efficacy of cold plasma in enhancing seed germination rates across various plant species, including tomatoes, wheat, and safflower. Similarly, the positive impact of PAW on germination has been reported, with increases ranging from 10 to 15 % in black gram seeds 21, 50 % in rye seeds, and even reaching 100 % germination in soybeans by the third day [24,25].

The maximum root and stem lengths of 5.05 cm and 9.02 cm, respectively, were observed in the seeds subjected to a 3-min DBD plasma treatment, followed by 3.63 pH of PAW priming (D3P2).

Fig. 15. A and B images related to the control group and C and D images related to the D3P2 treatment group (3 min of DBD treatment followed by priming in PAW with pH of 3.63). As can be seen in the photo, the treated seeds have better color and quality than the control group.

These findings are supported by previous studies [43,45,46,48,49] that have demonstrated the positive effects of plasma treatments on seedling growth and overall plant development. The improved wettability of the seeds, as evidenced by chemical and physical changes created on the seed surface and a significant increase in surface hydrophilicity (Figs. 10, 12 and 13), aligns with the observations of Bormashenko et al. [49], suggesting that plasma treatment can modify the chemical structure and surface roughness, thereby enhancing water absorption [50].

Moreover, the increased seedling dry weight by 53 % in D1P1(Fig. 8) observed in this study is proposed to be related to improved seed reserve mobilization and depletion percentage [51], as the interaction between seed cells and plasma may enhance the activities of germination enzymes and accelerate the breakdown of inner seed nutrients, contributing to improved seedling growth [52].

5. Conclusion

In conclusion, this study has demonstrated that subjecting May 344 cotton seeds to atmospheric DBD plasma followed by priming for 90 min in PAW with varying pH levels resulted in enhanced germination, germination rate, and seedling growth (Fig. 15). These findings suggest that cold plasma treatment can be effectively utilized to promote and improve the quality of cotton seeds.

Data availability statement

All relevant data are included within the article's figures. No additional data are available.

CRediT authorship contribution statement

Mohammad Hossein Mohajer: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Formal analysis, Data curation. **Motahare Monfaredi:** Software, Formal analysis. **Maede Rahmani:** Software, Formal analysis. **Mahdye Martami:** Writing – review & editing, Software, Methodology, Data curation, Conceptualization. **Elahe Razaghiha:** Software, Formal analysis. **Mohammad Hossein Mirjalili:** Writing – review & editing, Resources, Investigation. **Aidin Hamidi:** Writing – review & editing, Resources, Investigation, Conceptualization. **Hamid Reza Ghomi:** Resources, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] J. Snider, et al., Seed Size and Oil Content Are Key Determinants of Seedling Vigor in Gossypium Hirsutum, 2014.
[2] C. Alves Junior, et al., Water uptake mechanism and germination of Erythrina velutina seeds treated with atmospheric plasma, Sci. Rep. 6 (1) (2016) 33722.
[3] L. Ling, et al., Effects of cold plasma treatment on seed germination and seedling growth of soybean, Sci. Rep. 4 (1) (2014) 5859.
[4] G.J.J.B. de Groot, et al., Cold plasma treatment for cotton seed germination improvement, Sci. Rep. 8 (1) (2018) 14372.
[5] L. Sivachandiran, A. Khacef, Enhanced seed germination and plant growth by atmospheric pressure cold air plasma: combined effect of seed and water treatment, RSC Adv. 7 (4) (2017) 1822–1832.
[6] D. Butscher, et al., Plasma inactivation of microorganisms on sprout seeds in a dielectric barrier discharge, Int. J. Food Microbiol. 238 (2016) 222–232.
[7] S.-H. Ji, et al., Effects of high voltage nanosecond pulsed plasma and micro DBD plasma on seed germination, growth development and physiological activities in spinach, Arch. Biochem. Biophys. 605 (2016) 117–128.
[8] A. Zahoranová, et al., Effect of cold atmospheric pressure plasma on the wheat seedlings vigor and on the inactivation of microorganisms on the seeds surface, Plasma Chem. Plasma Process. 36 (2016) 397–414.
[9] J. Jiang, et al., Effect of seed treatment by cold plasma on the resistance of tomato to Ralstonia solanacearum (bacterial wilt), PLoS One 9 (5) (2014) e97753.
[10] J. Jiang, et al., Effect of cold plasma treatment on seed germination and growth of wheat, Plasma Sci. Technol. 16 (1) (2014) 54.
[11] S. Živković, et al., The stimulatory effect of non-equilibrium (low temperature) air plasma pretreatment on light-induced germination of Paulownia tomentosa seeds, Seed Sci. Technol. 32 (3) (2004) 693–701.
[12] F. Denes, S. Manolache, Y. Ra, Synthesis and surface functionalization under cold-plasma conditions, J. Photopolym. Sci. Technol. 12 (1) (1999) 27–38.
[13] J.C. Volin, et al., Modification of seed germination performance through cold plasma chemistry technology, Crop Sci. 40 (6) (2000) 1706–1718.
[14] Z. Giba, D. Grubišić, R. Konjević, Nitrogen oxides as environmental sensors for seeds, Seed Sci. Res. 13 (3) (2003) 187–196.
[15] D. Wendehenne, et al., Nitric oxide: comparative synthesis and signaling in animal and plant cells, Trends Plant Sci. 6 (4) (2001) 177–183.
[16] X.-Q. Wang, et al., Spectral characteristics of cotton seeds treated by a dielectric barrier discharge plasma, Sci. Rep. 7 (1) (2017) 5601.
[17] F.C. Fang, Antimicrobial reactive oxygen and nitrogen species: concepts and controversies, Nat. Rev. Microbiol. 2 (10) (2004) 820–832.
[18] B. Adhikari, et al., Cold plasma seed priming modulates growth, redox homeostasis and stress response by inducing reactive species in tomato (Solanum lycopersicum), Free Radic. Biol. Med. 156 (2020) 57–69.
[19] A. Waskow, A. Howling, I. Furno, Mechanisms of plasma-seed treatments as a potential seed processing technology, Frontiers in Physics 9 (2021) 617345.
[20] R.P. Guragain, et al., Influence of plasma-activated water (PAW) on the germination of radish, fenugreek, and pea seeds, AIP Adv. 11 (12) (2021).
[21] R.P. Guragain, et al., Effect of plasma treatment on the seed germination and seedling growth of radish (Raphanus sativus), Plasma Sci. Technol. 24 (1) (2021) 015502.
[22] V. Stoleru, et al., Plant growth promotion effect of plasma activated water on Lactuca sativa L. cultivated in two different volumes of substrate, Sci. Rep. 10 (1) (2020) 20920.
[23] S.A. Sajib, et al., Plasma activated water: the next generation eco-friendly stimulant for enhancing plant seed germination, vigor and increased enzyme activity, a study on black gram (Vigna mungo L.), Plasma Chem. Plasma Process. 40 (2020) 119–143.
[24] I. Naumova, A. Maksimov, A. Khlyustova, Stimulation of the germinability of seeds and germ growth under treatment with plasma-activated water, Surf. Eng. Appl. Electrochem. 47 (2011) 263–265.

[25] C.L. Porto, et al., Plasma activated water and airborne ultrasound treatments for enhanced germination and growth of soybean, Innovat. Food Sci. Emerg. Technol. 49 (2018) 13–19.
[26] D. Guo, et al., Plasma-activated water production and its application in agriculture, J. Sci. Food Agric. 101 (12) (2021) 4891–4899.
[27] W. Hoeben, et al., On the possibilities of straightforward characterization of plasma activated water, Plasma Chem. Plasma Process. 39 (2019) 597–626.
[28] M.M. Hefny, et al., Atmospheric plasma generates oxygen atoms as oxidizing species in aqueous solutions, J. Phys. Appl. Phys. 49 (40) (2016) 404002.
[29] T. Sarinont, et al., Plant growth enhancement of seeds immersed in plasma activated water, Mrs Advances 2 (18) (2017) 995–1000.
[30] W.-G. Ma, et al., Determination of tobacco (Nicotiana tabacum) seed vigour using controlled deterioration followed by a conductivity test, Seed Sci. Technol. 48 (2020).
[31] B. Abdallah, M. Kakhia, A. Obaide, Morphological and structural studies of ZnO nanotube films using thermal evaporation technique, Plasmonics 16 (5) (2021) 1549–1556.
[32] H. Krajian, et al., Hydrothermal growth method for the deposition of ZnO films: structural, chemical and optical studies, Microelectron. Reliab. 125 (2021) 114352.
[33] B. Abdallah, R. Hussin, W. Zetoune, Effect of etched silicon substrate on structural, morphological, and optical properties of deposited ZnO films via DC sputtering, Aerosol Science and Engineering 6 (1) (2022) 30–44.
[34] M. Abbasi Khalaki, A. Ghorbani, F. Dadjou, Influence of nano-priming on festuca ovina seed germination and early seedling traits under drought stress, in laboratory condition, Ecopersia 7 (3) (2019) 133–139.
[35] M.C. Pérez-Pizá, et al., Improvement of growth and yield of soybean plants through the application of non-thermal plasmas to seeds with different health status, Heliyon 5 (4) (2019) e01495.
[36] E. Arroyo, et al., Factors triggering germination in plasma-activated cotton seeds: water imbibition vs. reactive species' formation, J. Phys. Appl. Phys. 54 (32) (2021) 325205.
[37] R. Țiplea, et al., Alfalfa leaf powder and its potential utilisation in raw vegan chocolate, Bulletin UASVM Food Science and Technology 76 (2019) 1.
[38] X. Zhao, et al., Effect of superfine grinding on the physicochemical properties and antioxidant activity of red grape pomace powders, Powder Technol. 286 (2015) 838–844.
[39] R. Molina, et al., Influence of irrigation conditions in the germination of plasma treated Nasturtium seeds, Sci. Rep. 8 (1) (2018) 16442.
[40] Y. Gao, K. Francis, X. Zhang, Review on formation of cold plasma activated water (PAW) and the applications in food and agriculture, Food Res. Int. 157 (2022) 111246.
[41] Y. Meng, et al., Enhancement of germination and seedling growth of wheat seed using dielectric barrier discharge plasma with various gas sources, Plasma Chem. Plasma Process. 37 (2017) 1105–1119.
[42] Available from:: https://ijsst.areeo.ac.ir/.
[43] M. Dhayal, S.-Y. Lee, S.-U. Park, Using low-pressure plasma for Carthamus tinctorium L. seed surface modification, Vacuum 80 (5) (2006) 499–506.
[44] Z. Zhou, et al., Introduction of a new atmospheric pressure plasma device and application on tomato seeds, Agric. Sci. 2 (1) (2011) 23–27.
[45] Y. Meiqiang, et al., Stimulating effects of seed treatment by magnetized plasma on tomato growth and yield, Plasma Sci. Technol. 7 (6) (2005) 3143.
[46] M. Selcuk, L. Oksuz, P. Basaran, Decontamination of grains and legumes infected with Aspergillus spp. and Penicillum spp. by cold plasma treatment, Bioresour. Technol. 99 (11) (2008) 5104–5109.
[47] B. Sera, et al., Influence of plasma treatment on wheat and oat germination and early growth, IEEE Trans. Plasma Sci. 38 (10) (2010) 2963–2968.
[48] B. Šerá, et al., New physicochemical treatment method of poppy seeds for agriculture and food industries, Plasma Sci. Technol. 15 (9) (2013) 935.
[49] E. Bormashenko, et al., Cold radiofrequency plasma treatment modifies wettability and germination speed of plant seeds, Sci. Rep. 2 (1) (2012) 741.
[50] S. Wild, L. Kesmodel, High resolution electron energy loss spectroscopy investigation of plasma-modified polystyrene surfaces, J. Vac. Sci. Technol. A: Vacuum, Surfaces, and Films 19 (3) (2001) 856–860.
[51] A. Soltani, M. Gholipoor, E. Zeinali, Seed reserve utilization and seedling growth of wheat as affected by drought and salinity, Environ. Exp. Bot. 55 (1–2) (2006) 195–200.
[52] D. Dobrynin, et al., Physical and biological mechanisms of direct plasma interaction with living tissue, New J. Phys. 11 (11) (2009) 115020.