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Research article

Optimizing beet seed germination via dielectric barrier discharge plasma parameters

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ABSTRACT

This study explores the synergistic effects of gas composition and electric field modulation on beetroot seed germination using dielectric barrier discharge (DBD) plasma. The investigation initially focuses on the impact of air plasma exposure on germination parameters, varying both voltage and treatment duration. Subsequently, the study examines how different gas compositions (argon, nitrogen, oxygen, and carbon dioxide) affect germination outcomes under optimal air plasma conditions. Results indicate that plasma treatment significantly enhances germination rates and seedling growth relative to untreated controls. Notably, plasma exposure alters seed surface morphology and chemistry, increasing roughness, porosity, and hydrophilicity due to the formation of new polar functional groups. The highest germination rate (a 54.84 % increase) and germination index (a 40.11 % increase) were observed at the lowest voltage and shortest duration, whereas higher voltages and prolonged exposure reduced germination, likely due to oxidative stress. Among the tested gas environments, air plasma was most effective in enhancing water uptake and electrical conductivity, while oxygen plasma resulted in the highest germination index and marked improvements in root and shoot length. Conversely, carbon dioxide plasma treatment exhibited inhibitory effects on both germination and subsequent growth metrics. The results highlight the potential of DBD plasma technology to enhance agricultural productivity by optimizing seed germination and early growth. The study emphasizes the importance of precise parameter tuning, particularly gas composition and plasma exposure conditions, to maximize benefits while minimizing adverse effects, offering a refined approach to seed priming in agricultural practices.

1. Introduction

Agriculture, a fundamental pillar of our civilization, plays a vital role in supplying food, fiber, and energy to the growing population, supporting rural economies and contributing to national and global food security [1]. In this sector, effective seed germination

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being a critical determinant of crop yields and maintaining agricultural productivity [2]. Successful seed germination leads to the development of healthy seedlings with vigorous root and stem formation, which are essential for optimal plant growth and productivity [3]. Adequate germination rates are critical for successful crop establishment, as insufficient germination can result in reduced yields and financial setbacks for agricultural producers [4]. Researchers have explored various techniques to improve seed germination rates and enhance crop productivity [5]. Among these strategies, plasma treatment has emerged as an innovative and environmentally friendly approach that has gained considerable attention. This technique shows great promise in enhancing agricultural practices while simultaneously minimizing the environmental footprint of crop production [6,7].

Plasma, the fourth state of matter, consists of highly energized particles generated by applying an electric field to a gas or liquid, which produces a variety of reactive species [8]. Research has demonstrated that these reactive species exert positive effects on seed germination by inducing a range of physiological and biochemical changes within seeds [9]. Plasma-generated reactive species trigger a cascade of cellular responses, including enzyme activation, alteration of seed surface properties, and stimulation of cell division and elongation. Collectively, these responses contribute to enhanced seed germination and seedling establishment [10–12]. Moreover, plasma treatment not only enhances initial growth stages but also augments nutrient uptake and fortifies plants against various environmental stresses and pathogens [13,14].

Beta vulgaris, commonly known as beetroot, is an economically significant crop due to its distinctive pigments and exceptional nutritional profile, making it valuable in diverse culinary, industrial, and health-related applications [15]. Despite its agricultural and economic significance, beetroot seed germination faces a complex array of intrinsic and extrinsic obstacles that significantly impact crop establishment and overall agricultural productivity [16,17]. These germination challenges are predominantly attributed to factors such as the presence of rigid seed coats, which impede water absorption, low seed coat permeability, and intrinsic dormancy mechanisms. These factors can lead to suboptimal germination rates and poor seedling emergence, thereby compromising the effectiveness of crop establishment and ultimately diminishing the productivity of beetroot cultivation [18,19]. To address these obstacles, researchers have been conducting extensive studies aimed at identifying and developing innovative strategies to enhance seed germination and improve overall crop performance [19–22]. These efforts are crucial for advancing beetroot cultivation practices and ensuring sustainable production to meet growing global demand.

Plasma treatment has recently emerged as a promising technique for enhancing seed germination and seedling growth across various crops [23–25]. However, despite its potential, the application of plasma technology for optimizing beetroot seed germination remains largely unexplored. This research gap presents an opportunity to explore the potential of plasma treatment as a practical solution to improve beetroot seed germination and contribute to the advancement of sustainable agricultural practices. The present study seeks to address the challenges associated with beetroot seed germination by investigating the potential of dielectric barrier discharge (DBD) plasma technology as an innovative and eco-friendly strategy. This research specifically examines the effects of DBD plasma treatment on beetroot seed germination, with the ultimate goal of advancing agricultural practices and promoting enhanced crop productivity. To mitigate the risk of seed damage and to ensure a uniform treatment environment, the study employed an innovative approach involving the electric field modulation using dual power sources to generate a homogeneous plasma. This approach effectively minimizes the possibility of localized overheating and uneven treatment, thereby enhancing the reliability and reproducibility of the plasma treatment process. This study is structured in two distinct phases. The first phase investigates the impact of air plasma exposure on germination rates and subsequent seedling development of beetroot. Building upon these findings, the second phase explores how modifying the gas composition within the optimized air plasma treatment parameters influences germination outcomes. A comprehensive analysis of plasma-induced changes was performed using a multi-faceted approach. The surface morphology of the seeds was examined using scanning electron microscopy (SEM), which provided detailed insights into the structural alterations. Concurrently, chemical modifications were assessed through Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR), enabling the identification of molecular changes. Additionally, plasma characteristics were examined through optical emission spectroscopy (OES), and these data were correlated with the observed effects on seed germination and seedling growth. The success of germination was quantitatively assessed through germination rates, root lengths, and stem lengths growth across different treatment conditions. Additionally, the soaking capacity of seeds was measured to gain insights into potential changes in seed coat permeability.

This comprehensive investigation aims to contribute to our understanding of plasma-seed interactions and their potential applications in agriculture. By elucidating the mechanisms underlying plasma-induced enhancements in seed germination and seedling vigor, we hope to pave the way for innovative, sustainable approaches to improving crop yields and food security.

2. Material and methods

2.1. Beetroot seeds

The monogerm beetroot seeds (*Beta vulgaris*) were procured from the Seed and Plant Certification and Registration Institute (Karaj, Iran). The seeds underwent a series of preparation steps to ensure consistent surface properties and minimize experimental variability. First, seeds were selected based on size and morphology. Only seeds exhibiting a distinct star shape and weighing between 10 and 12 mg were selected. Selected seeds were then cleaned to remove surface contaminants. This involved washing with a mild detergent solution followed by thorough rinsing with deionized water. Finally, seeds were dried in a temperature-controlled oven at 30 °C for 24 h to remove residual moisture, a process chosen based on a literature review and validated by preliminary experiments [26–28]. Following the drying process, the prepared seeds were transferred to the plasma chamber for plasma treatment.

2.2. Enhanced dielectric barrier discharge plasma

The plasma generation chamber consists of a cylindrical quartz glass container with internal dimensions of 22 cm in diameter, 9 cm in height, and a wall thickness of 5 mm. The device is equipped with a pair of parallel stainless-steel electrodes, with the inter-electrode distance being precisely adjustable. Plasma generation is facilitated by a dual-mode power system, comprising two high-voltage power supplies operating at frequencies of 50 Hz and 6 kHz, with voltage output ranges of 0–25 kV and 0–15 kV, respectively [29,30]. As depicted in Fig. 1, the upper electrode is connected to a 6 kHz direct current (DC) pulsed power supply, while the lower electrode is connected to a 50 Hz sinusoidal power source. Throughout the experiments, a fixed inter-electrode distance of 15 mm was maintained between the upper electrode and the base of the quartz chamber to ensure uniform plasma exposure. Additionally, a constant voltage of 12 kV was applied to the upper electrode throughout the experimental procedures. The current and voltage profiles applied to the electrodes are illustrated in Fig. 2, which shows the waveforms for the three different power supply configurations: the 6 kHz pulsed power supply (Fig. 2a), the 50 Hz sinusoidal power supply (Fig. 2b), and the combined amplitude-modulated power supply (Fig. 2c), achieved by simultaneously applying both power supplies.

The study investigated two independent variables: the treatment duration and the voltage applied to the lower electrode. Treatment duration was tested at three discrete levels: 1, 3, and 5 min. The voltage applied to the lower electrode was varied across three distinct levels: 14, 19, and 24 kV. The mean electrical power delivered to the electrodes was calculated through integration of the product of the instantaneous voltage and current over one or more complete cycles of the waveform [31]. Voltage measurements are presented as peak values (Vp), indicating the maximum voltage applied during each cycle, as measured by a TEKTRONIX P6015 high voltage probe. Current measurements were obtained with a TCP202 TEKTRONIX current probe, with both signals monitored on a TEKTRONIX DPO 3012 oscilloscope. A consistent power input of 83.28 W was maintained for the upper electrode throughout the experiment, while the average electrical powers applied to the lower electrode were calculated to be 5.28 W, 9.12 W, and 12.86 W at 14 kV, 19 kV, and 24 kV, respectively. The presence of metastable plasma species was investigated using the AvaSpec-ULS2048CL-EVO optical emission spectrometer. Radial spectroscopic measurements were obtained at a distance of 25 mm from the plasma source and 8 mm from the container endpoint. In each treatment, a single layer of seeds was placed uniformly within the chamber to ensure consistent exposure to the plasma.

2.3. Seed treatment

The seed treatment protocol consists of two distinct experimental phases. The primary objective of the first phase was to investigate the effect of air plasma on beetroot seed germination. Ten treatment groups were established, with one serving as the control group and the remaining nine groups subjected to plasma treatment, as detailed in Table 1. Following the identification of the optimal treatment group in the initial experiment, a subsequent phase was conducted to examine the effects of different plasma gas combinations on seed germination and growth. This phase utilized argon, nitrogen, oxygen, and carbon dioxide gases, each applied at a constant flow rate of 5 standard liters per minute (SLM). The aim of this experiment was to explore potential synergistic effects while maintaining the optimal conditions identified in the initial phase. Each treatment was replicated three times, with a total of 50 seeds utilized in each replicate. Post-treatment, the seeds were arranged in groups of ten within sterilized petri dishes lined with autoclaved Whatman filter paper. The seeds were then moistened with 1 mL of autoclaved distilled water and transferred to a germinator maintained at a temperature of 15 °C and a relative humidity of 71–75 %. Throughout the growth phase, additional distilled water was periodically added to the petri dishes as needed.

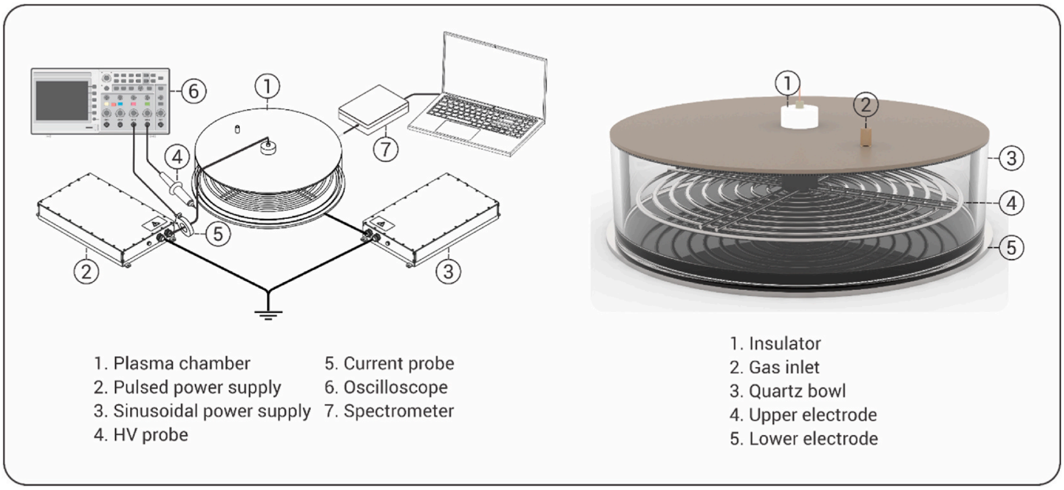

Fig. 1. Schematic representation of the experimental setup for DBD, illustrating the arrangement of electrodes, power supply, and data acquisition system.

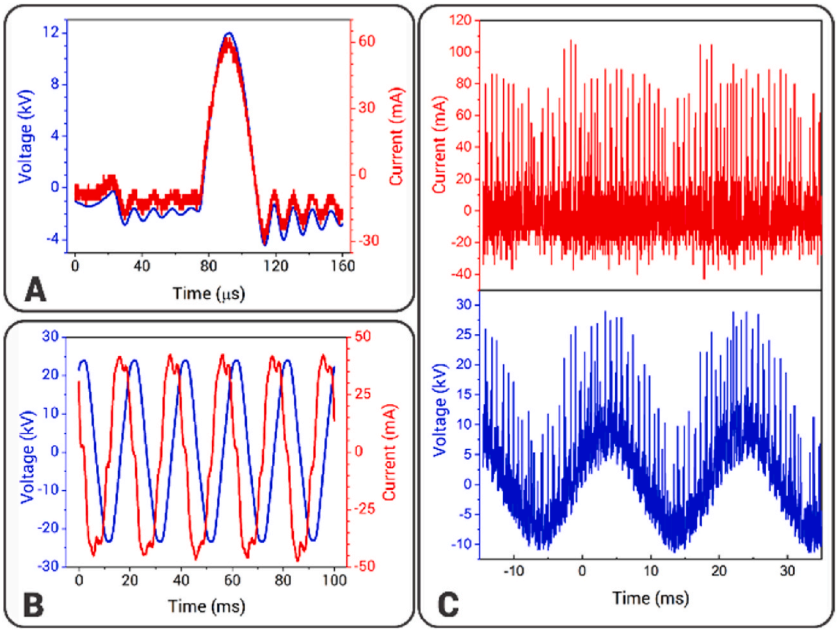

Fig. 2. Current and voltage waveforms of three power supply configurations: A) 6 kHz pulsed power supply, B) 50 Hz sinusoidal power supply, and C) combined amplitude-modulated power supply, achieved by simultaneously applying both power supplies with a 14 kV voltage applied to the sinusoidal power supply.

Table 1
Overview of seed classifications according to pre-sowing treatment protocols.

Group	Description
C	Control seeds
P141	Plasma treated seed (14 kV, 1 min)
P143	Plasma treated seed (14 kV, 3 min)
P145	Plasma treated seed (14 kV, 5 min)
P191	Plasma treated seed (19 kV, 1 min)
P193	Plasma treated seed (19 kV, 3 min)
P195	Plasma treated seed (19 kV, 5 min)
P241	Plasma treated seed (24 kV, 1 min)
P243	Plasma treated seed (24 kV, 3 min)
P245	Plasma treated seed (24 kV, 5 min)

2.4. Seed germination

Germination progress was monitored over a 14-day period in a dark germinator, with daily observations and records of the number of germinated seeds. The germination percentage and germination index were subsequently calculated according to established protocols [32,33]. Germination kinetics were assessed by fitting the experimental data to Richard's curve [34], a model frequently employed to characterize seed germination patterns. At the conclusion of the 14-day germination period, seedlings were carefully removed from the germinator, and morphometric measurements were conducted to quantify the average lengths of both roots and stems.

2.5. Seed imbibition

The water absorption capacity of the seeds was assessed by determining the percentage of water uptake, calculated by measuring the initial dry weight of the seeds and their subsequent weight after soaking in water [32,33]. For each replicate, 50 seeds were used, and the experiment was conducted in three independent replicates. The seeds were placed in sterile plastic containers, each containing 50 mL of deionized water, and incubated at a temperature of 25 ± 1 °C in a temperature-controlled laboratory environment. Each experiment was conducted on a laboratory bench equipped with centralized temperature monitoring to ensure environmental consistency. After a 24-h incubation period, the saturated mass of the seeds was measured. The seeds were then subjected to a drying process to obtain their final dry weights, and the water uptake was subsequently calculated. Additionally, the electrical conductivity of the soaking solution was measured using an Istek desktop multiparameter benchtop water quality meter (Model PDC-700L) to assess ion leakage from the seeds. This measurement serves as an indicator of changes in seed coat permeability and potential effects of plasma treatment on seed integrity and subsequent germination vigor [35,36].

2.6. Seed characteristics

The surface morphology of both untreated and plasma-treated seeds was examined using a Hitachi SU3500 SEM. To assess changes in the surface chemical structure of the seeds before and after plasma treatment, ATR-FTIR spectroscopy was performed. ATR-FTIR spectra were acquired using a NEXUS 470 ATR-FTIR spectrometer over a wavenumber range of 4000–500 cm^{-1}, utilizing 60 scans at a resolution of 4 cm^{-1}. he wettability of the seeds was evaluated using the sessile drop method to measure the static contact angle of deionized water droplets on the seed surface. For each measurement, 2 μL of deionized water was dispensed onto the seed surface. Images were captured using a charge-coupled device (CCD) camera.

2.7. Statistical analysis

Experimental data were collected in triplicate, and results were expressed as the mean ± standard deviation (SD). Statistical significance was determined using a one-way analysis of variance (ANOVA) with a significance level set at $P < 0.05$. Multiple comparison tests were performed using Tukey's HSD to identify significant differences between groups.

3. Results

3.1. Optical emission spectrum

The optical emission spectrum of the air plasma, displayed in Fig. 3a, was obtained by exposing the plasma to the seed surface. The spectral lines identified in the air plasma and their corresponding species are detailed in Table 2. Notably, an increase in the voltage applied to the bottom electrode in the plasma reactor resulted in enhanced energy deposition, thereby generating a more energetic plasma. This is evidenced by the increasing ratio of the oxygen peak intensity at 777 nm to nitrogen peak intensity at 337 nm (Fig. 3b), indicating a relative increase in the abundance of oxygenated species, including atomic oxygen, oxygen radicals, and ozone, compared to nitrogen-containing species.

The presence of reactive oxygen species (ROS) in plasma treatments has been demonstrated to stimulate the activity of critical enzymes, such as α-amylase and protease, which are essential for carbohydrate and protein breakdown in beetroot seeds [37,38]. This enhanced enzymatic activity therefore improves nutrient availability for the developing embryo, thereby promoting seed germination and early seedling growth [39]. Furthermore, ROS regulate the expression of genes associated with cell wall loosening and expansion, facilitating radicle emergence and seedling establishment [8,40,41]. In addition, ROS have been shown to enhance plant tolerance to abiotic stress by bolstering the antioxidant defense system [42]. The presence of hydroxyl radicals in the plasma spectra suggests a potential role in the oxidation of biomolecules on the beet seed surface, which may modify seed coat properties, enhance permeability, and increase water uptake, thereby facilitating seed imbibition and accelerating the germination process [43,44]. However, excessive levels of ROS can have detrimental effects on germination, including oxidative damage to cellular components [45]. Moreover, reactive nitrogen species (RNS) also contribute to seed coat modifications, improving permeability and fostering germination, growth, and agricultural productivity [41,46]. Nitric oxide (NO), a key RNS, acts as a signaling molecule, interacting with hormonal pathways (e.g., abscisic acid (ABA) and gibberellins (GA)) that regulate seed dormancy and germination [37]. NO also modulates antioxidant

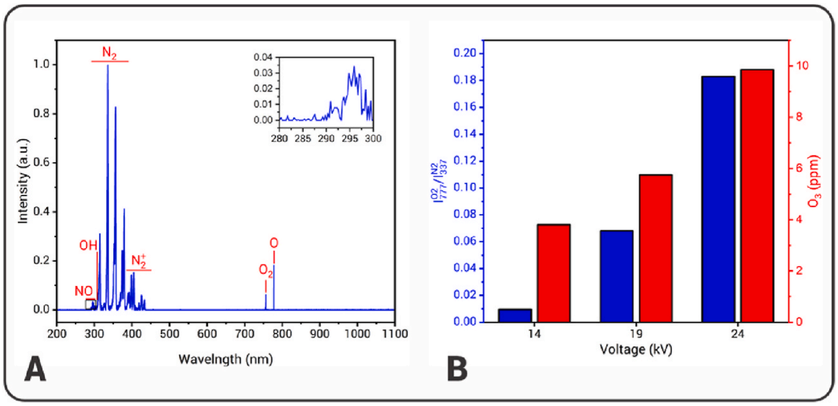

Fig. 3. A) Air plasma spectrum obtained at 25 mm from the plasma chamber outlet after a 3-min exposure to a 19 kV discharge. B) Ratio of oxygen peak intensity at 777 nm to nitrogen peak intensity at 337 nm plotted against applied voltage (kV). Ozone concentration (ppm) measured in the plasma chamber is also shown.

Table 2
Spectral lines identified in air plasma and their corresponding species.

Species	Transitions	Peak positions (nm)	Ref.
NO_γ	$A^2\Sigma^+ \rightarrow X^2\Pi_r$	280–300	[24,65]
OH	$A^2\Sigma^+ \rightarrow X^2\Pi_{3/2}$	309	[66]
N_2 (SPS)	$C^3\Pi \rightarrow B^3\Pi$	315.9; 337.1; 357.7; 380.5	[24]
N_2^+ (FNS)	$B^2\Sigma_u \rightarrow X^2\Sigma_g$	427.8; 434.2; 441.7	[24]
O_2	A-band	756	[67]
O	3p→3s	740–845	[68]

enzyme activity, thereby maintaining redox balance in beetroot seeds [47].

3.2. Physicochemical properties of beet seeds

The physicochemical properties of beet seeds were significantly altered following air plasma treatment, as evidenced by SEM and ATR-FTIR spectroscopy. While electrical conductivity is reported as a physical property to assess membrane integrity, the chemical properties were assessed using ATR-FTIR spectroscopy, which provides insights into molecular-level changes on the seed surface. SEM analysis of the seed coat surfaces (Fig. 4) revealed pronounced morphological changes, characterized by increased surface roughness, enhanced porosity, and the formation of microgrooves. These changes are evident when comparing the untreated control sample (Fig. 4a) to the seeds subjected to 3 min of air plasma treatment (Fig. 4b). These surface modifications are attributable to the combined effects of etching and ablation processes induced by the plasma treatment. The resulting topographical changes are hypothesized to improve seed imbibition and nutrient uptake, thereby potentially enhancing the germination process [48].

The ATR-FTIR spectrum of the seeds (Fig. 5) reveals a complex array of vibrational modes characteristic of various functional groups. The spectrum features prominent peaks corresponding to O-H stretching vibration (3000–3500 cm^{-1}), C-H and CH_2 bending vibrations (1326 and 1420 cm^{-1}), C-H stretching vibration (2932 and 2872 cm^{-1}), double C=O stretching vibration (1650–1750 cm^{-1}), and C-O stretching vibrations (1233 and 1093 cm^{-1}) [49]. Following plasma treatment, significant alterations are observed in the ATR-FTIR spectrum, particularly in the regions corresponding to O-H, C-O, and C=O functionalities. These changes are evidenced by variations in peak intensities and shifts in wavenumbers, indicating modifications in oxygen-containing functional groups on the seed surface [50]. Furthermore the plasma treatment induces alterations in the vibrational characteristics of C-H bonds, as demonstrated by shifts in peak positions and changes in peak intensities. These modifications suggest the formation of intermolecular hydrogen bonds and the introduction of polar functional groups, which likely contribute to the oxidation of C-H bonds, the degradation or removal of germination inhibitors, and an increase in seed coat hydrophilicity. These effects collectively enhance the potential for seed germination [51–53].

The static contact angle measurements and corresponding images of water droplets on the surface of beet seeds subjected to DBD

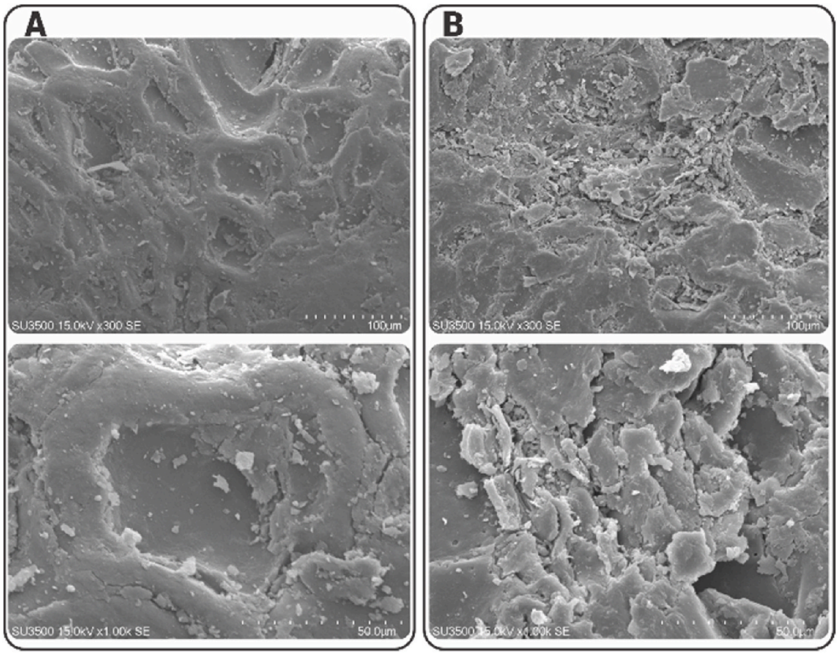

Fig. 4. SEM images of the surface topography of beet seeds: A) untreated control sample and B) after 3 min of air plasma treatment.

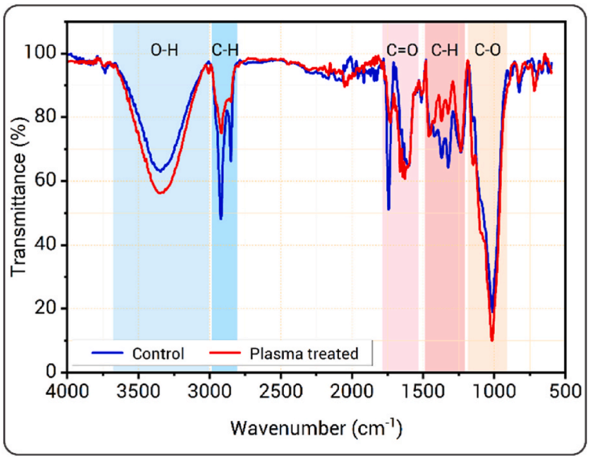

Fig. 5. The ATR-FTIR spectra of beet seeds exposed to air plasma treatment, contrasted with those of untreated control seeds.

plasma treatment are presented in Fig. 6. The plasma treatment results in a significant reduction in the contact angle of water droplets on the seed surface, indicative of increased hydrophilicity. This change is attributed to the physical and chemical modifications induced by the plasma treatment, including surface etching and functionalization. The introduction of new polar functional groups, such as hydroxyl and carboxyl groups, on the seed surface enhances surface energy and wettability, as evidenced by the observed decrease in the contact angle [54].

3.3. Germination and vigor

The effects of air DBD plasma treatment on beetroot seed germination under varying conditions are presented in Fig. 7. The influence of the applied voltage on the daily germination rate is shown for 14 kV (Fig. 7a), 19 kV (Fig. 7b), and 24 kV (Fig. 7c), with treatment durations of 1 min (red), 3 min (green), and 5 min (blue) indicated by different color schemes. The plasma-treated groups generally exhibited a significantly enhanced germination rate compared to the control group. Notably, a 1-min treatment duration yielded the highest germination rate. However, extending the treatment time to 3 and 5 min resulted in a decline in germination, suggesting a potential time-dependent sensitivity of seeds to plasma exposure. Moreover, an inverse relationship was observed between plasma voltage and germination rate at fixed treatment times, with increasing voltage corresponding to decreased germination. The observed decline in germination rate with increasing plasma voltage suggests a negative correlation between plasma intensity and seed viability. This decline aligns with the increased generation of ROS by plasma treatment. While ROS play a role in seed germination signaling, excessive ROS production, as would occur with increased plasma voltage, can induce oxidative stress within beetroot seeds, eading to the degradation of essential biomolecules and cellular structures, thereby compromising seed viability [55,56].

The Richard curve was fitted to the daily germination data to model the germination kinetics of seeds subjected to air DBD plasma treatment, as depicted in Fig. 8. The analysis of Richard's curves revealed distinct germination patterns under varying plasma treatment conditions. The plasma-treated seeds exhibited a significantly shorter lag time compared to the control group, suggesting an accelerated onset of germination. This observation aligns with the hypothesis that reactive oxygen species (ROS) modulate the balance between abscisic acid (ABA) and gibberellic acid (GA). Specifically, ROS may reduce ABA levels while enhancing GA biosynthesis or signaling pathways, thereby facilitating the breakdown of dormancy and promoting germination [57–59]. During the exponential phase of germination, the treatments P141, P143, P191, and P193 demonstrated steeper slopes than the control, suggesting a faster

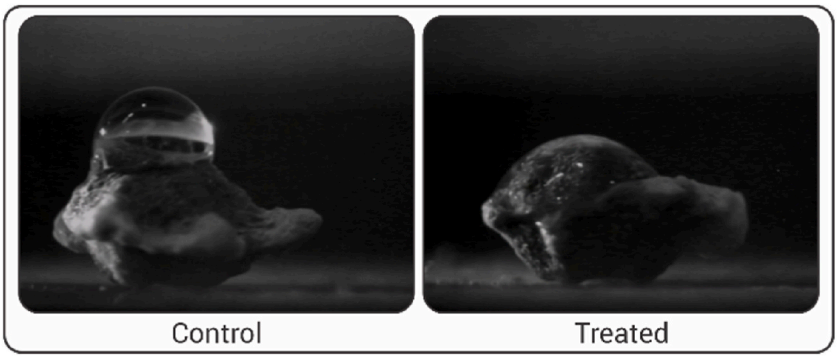

Fig. 6. Impact of air plasma exposure on beet seed hydrophilicity: contact angle measurements before and after 3 min of treatment.

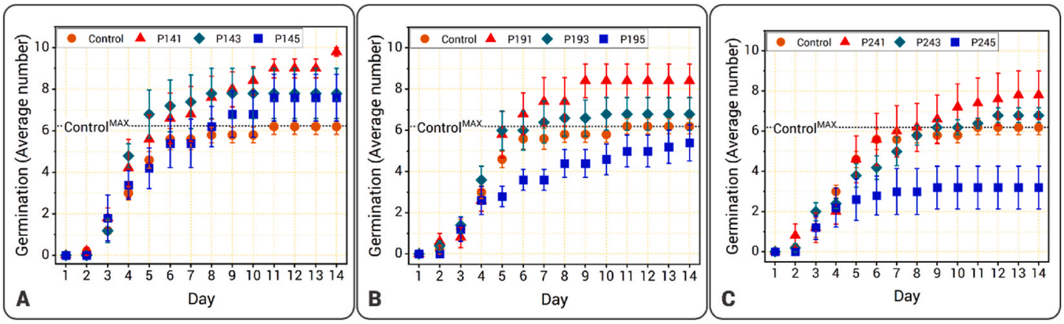

Fig. 7. Influence of applied voltage and treatment duration on the daily germination rate of beetroot seeds subjected to air plasma treatment. The responses of seed germination to varying voltage levels are depicted in A) 14 kV, B) 19 kV, and C) 24 kV, with treatment durations of 1 min (red), 3 min (green), and 5 min (blue) denoted by distinct color schemes.

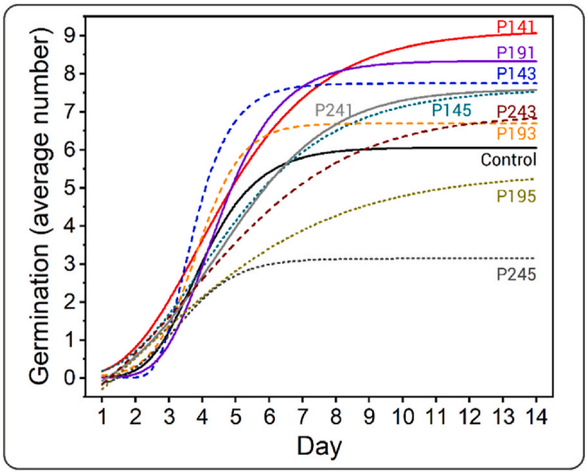

Fig. 8. Dynamics of seed germination post-plasma treatment: a richards curve analysis showing progression towards maximum germination potential.

germination rate [46]. In contrast, seeds exposed to higher voltages and longer treatment durations displayed flatter slopes throughout the germination period, which may be attributed to excessive oxidative damage and thermal stress [60,61]. In the final stage of germination, the Richard curves plateaued, representing the maximum germination capacity (upper asymptote) for each treatment group. The control group exhibited a mean upper asymptote of 6.2 ± 0.37 germinated seeds per replicate, whereas the plasma-treated groups displayed a range of values, with median values ranging from 3.2 ± 1.07 (P245) to 9.8 ± 0.2 (P141) germinated seeds per replicate. These findings highlight the differential effects of treatment parameters on seed germination. A significant negative correlation was observed between treatment parameters (voltage and duration) and germination rate, as indicated by the daily germination data. Specifically, seeds treated with plasma at higher voltages and longer durations exhibited a marked inhibition of germination, with a significant reduction in germination rate compared to the control group. The mean reduction in germination rate was 15 % (95 % CI: 10–20 %) for the P195 treatment and 38 % (95 % CI: 30–46 %) for the P245 treatment.

The germination results (Fig. 9) demonstrate that plasma treatment has a significant impact on both germination percentage (GPP) and germination index (GI). The P141 group exhibited the highest GPP (96 %) and GI (2.005), both of which were significantly higher than those of the control group (GPP = 62 %, GI = 1.431) ($p < 0.05$). While other treatment groups, including P143, P145, P191, P193, and P241, also showed higher GPP and GI compared to the control, not all differences reached statistical significance. Comparative analysis of the plasma-treated groups indicates that applied voltage and treatment duration significantly influence GI and GPP. Among the voltage levels tested, the 14 kV groups (P141, P143, and P145) generally outperformed the 19 kV and 24 kV groups in terms of GPP and GI. Specifically, the 14 kV groups exhibited significantly higher GPP and GI values than the 24 kV groups (P243 and P245) ($p < 0.05$). Within the 14 kV groups, the P141 group (1-min treatment) showed the highest GPP and GI, followed by the P143 (3-min) and P145 (5-min) groups. Regarding treatment duration, the results suggest that shorter treatment times are more effective in enhancing germination. The 1-min treatment groups (P141 and P191) had significantly higher GPP and GI than the 5-min treatment groups (P145 and P245) ($p < 0.05$). Furthermore, the 3-min treatment groups (P143 and P193) exhibited intermediate GPP and GI values. These findings suggest that the optimal conditions for enhancing the germination of beetroot seeds are achieved by applying the minimum voltage and shortest treatment duration.

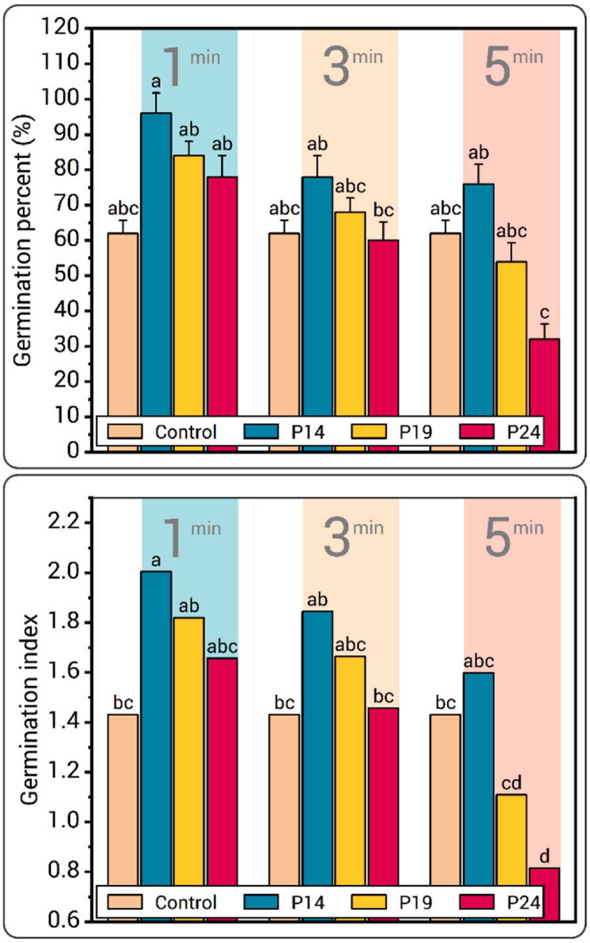

Fig. 9. Germination percent and germination index of seeds are shown in response to air plasma treatment. Data are presented as a mean value ± standard deviation. The different superscript letters are significant ($p < 0.05$).

The effect of DBD plasma treatment under different gas environments on seed germination and early growth was investigated, building on the optimal air plasma conditions established in the previous phase (Fig. 10). The results showed that air plasma treatment yielded the highest GPP of 96 % (Fig. 11a), which was significantly higher than that of the other treatments ($p < 0.05$). In contrast, oxygen plasma treatment resulted in the highest GI of 2.919, root length of 35 mm, and stem length of 54.67 mm compared to all other treatments ($p < 0.05$) (Fig. 11b). These findings reveal a positive correlation between the elevated levels of the ROS generated through plasma seed treatment in air and oxygen environments and the subsequent enhancement of seed germination and early growth, underscoring the superior efficacy of this treatment modality relative to other plasma-based approaches. Furthermore, exposure to carbon dioxide (CO_2) and argon plasma did not significantly improve either GPP or GI compared to the control or other treatments. Notably, CO_2 plasma treatment yielded the lowest GPP (36 %), GI (1.444), and root length (11 mm), indicating a potentially detrimental effect on these parameters. This suggests that the reactive species generated in CO_2 plasma may disrupt crucial metabolic processes, such as carbon and nitrogen metabolism, essential for energy production and biosynthesis during germination and early growth. Imbalances in these pathways could lead to reduced resource availability and the accumulation of toxic metabolites, hindering plant development [62,63].

The impact of DBD plasma treatment on seed water uptake and electrical conductivity (EC) is illustrated in Fig. 12, demonstrating a significant dependence on the gas composition employed. Seeds treated with air plasma exhibited the highest water uptake (43 %), significantly surpassing the control (37 %) and all other gas treatments except for oxygen (40 %) ($p < 0.05$). This enhanced water absorption can be attributed to the generation of ROS during air plasma treatment, which are known to modify seed coat structure and increase permeability [64]. Furthermore, air plasma treatment resulted in significantly higher EC (430 μS/cm) compared to the control (390 μS/cm) ($p < 0.05$), potentially reflecting greater leakage of ions and metabolites due to plasma-induced modifications in seed coat and cellular membrane permeability [35]. In contrast, argon plasma treatment resulted in the lowest EC (397 μS/cm), suggesting minimal disruption to seed ionic balance and membrane integrity.

The observed variations in germination and growth parameters among the various gas plasma treatments can be attributed to the distinct chemical compositions and reactive species generated within each plasma environment. The presence of reactive species,

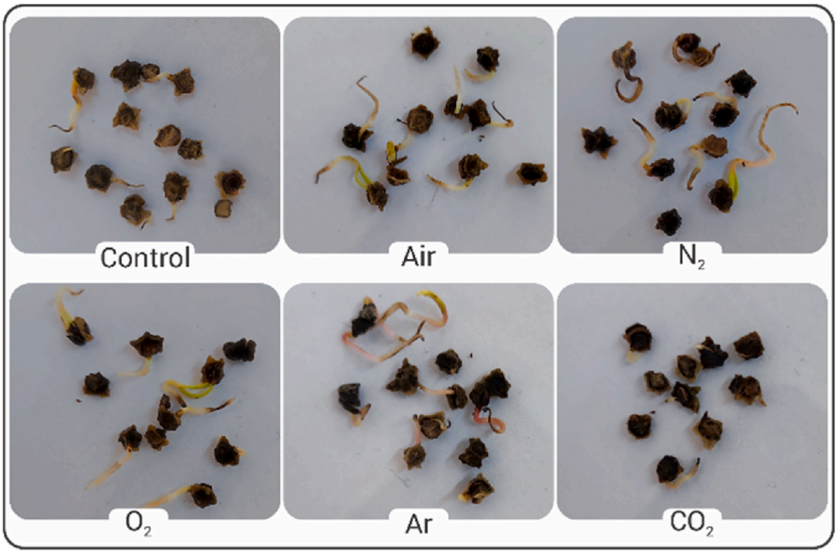

Fig. 10. Seed germination responses to atmospheric pressure plasma treatment, with gas composition as a variable. Seeds were treated with plasma generated using different gases (N_2, O_2, argon, and CO_2) under optimized conditions for air plasma treatment.

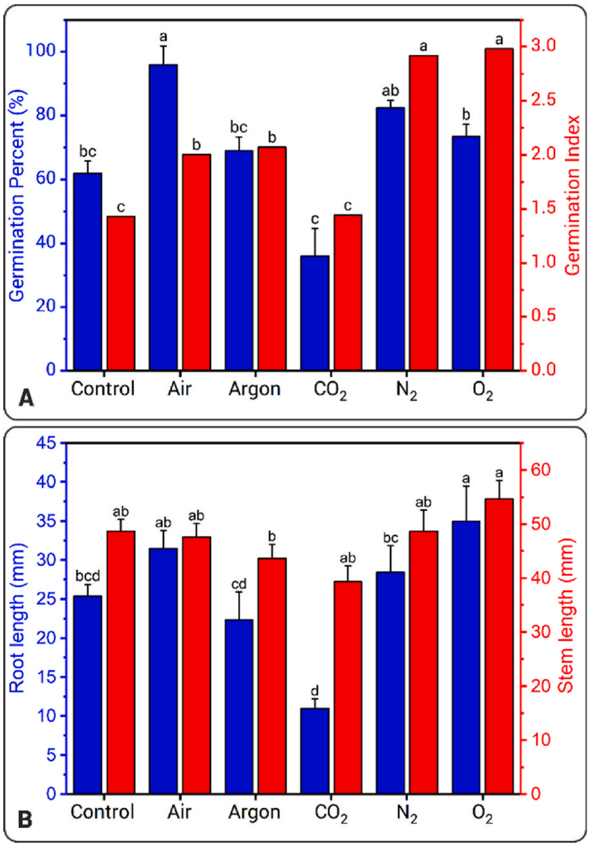

Fig. 11. A) effect of different gas plasma treatments on germination metrics: germination percentage and index, B) growth response in terms of root and stem length in beetroot seeds following plasma exposure. Data are presented as a mean value ± standard deviation. The different superscript letters are significant ($p < 0.05$).

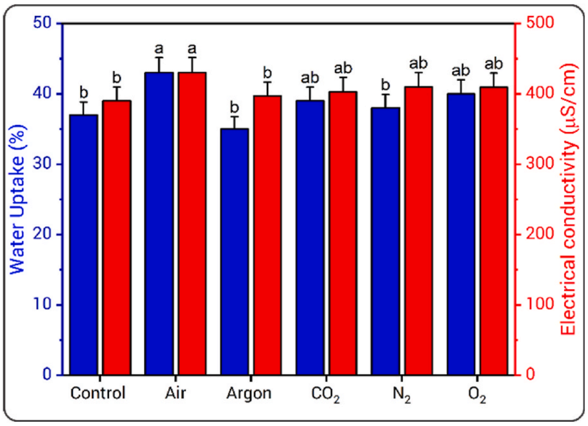

Fig. 12. DBD plasma treatment effects on beetroot seed electroconductivity and water uptake. Seeds were exposed to plasma treatment for 1 min at an applied voltage of 14 kV. Data are presented as a mean value ± standard deviation. The different superscript letters are significant ($p < 0.05$).

including oxygen and nitrogen radicals, within the plasma induces physiological responses in the seeds, leading to enhanced germination and growth outcomes [52]. The specific composition and concentration of these reactive species in different gas plasmas likely contribute to the observed heterogeneity in germination and growth parameters.

4. Conclusion

This study provides a comprehensive investigation into the effects of DBD plasma treatment on the germination and early growth of beetroot seeds. Our findings demonstrate that DBD plasma treatment significantly influences seed germination, morphology, and biochemical properties, with notable variations observed based on treatment duration, voltage, and gas composition. SEM and ATR-FTIR analyses revealed significant plasma-induced changes on the seed surface, including increased roughness, enhanced porosity, and alterations in functional groups. These modifications improve seed coat permeability and water uptake, which are critical factors in promoting germination. The optimal conditions for maximizing GPP and GI were identified as a 1-min treatment duration at 14 kV, which significantly outperformed the control group, yielding a GPP of 96 % and a GI of 2.005, compared to 62 % and 1.431 for the control, respectively. However, prolonged treatment durations and higher voltages were found to negatively impact germination rates, likely due to excessive oxidative stress and thermal damage. This underscores the dual role of ROS and RNS in seed germination: while moderate levels can enhance enzyme activities, modify seed coat properties, and facilitate water uptake, excessive levels can induce oxidative damage, thereby reducing seed viability.

The study also highlights the differential impacts of various plasma gases, with air and oxygen plasmas being particularly effective in enhancing seed germination and early growth parameters. Notably, oxygen plasma treatment achieved the highest GI and significant increases in root and stem lengths, emphasizing the potential for specific gas environments to optimize plasma treatment outcomes.

In conclusion, DBD plasma treatment emerges as a potent and eco-friendly strategy to improve beetroot seed germination and early growth. The optimal conditions identified in this study offer a promising approach to enhancing agricultural productivity sustainably. Future research should focus on scaling up this technology and exploring its applicability across diverse crop species to further advance sustainable agricultural practices.

CRediT authorship contribution statement

Mohammad Hossein Mohajer: Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ahmad Khademi:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maede Rahmani:** Formal analysis. **Motahare Monfaredi:** Investigation, Formal analysis. **Aidin Hamidi:** Writing – review & editing, Supervision, Resources. **Mohammad Hossein Mirjalili:** Writing – review & editing, Supervision, Resources. **Hamid Ghomi:** Writing – review & editing, Supervision, Resources, Methodology.

Data and code availability

Data included in article/supplementary material is referenced in the article.

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.

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