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RESEARCH ARTICLE

Electrohydraulic Discharge Induced Gas-Liquid Interface Plasma for Seed Priming in Hydroponics

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ABSTRACT Seed priming is a vital process in agriculture, improving germination and uniformity. This study presents an innovative seed priming approach using electrohydraulic discharge plasma (EHDP) techniques, inducing gas-liquid interface plasma on wet seeds—EHDP-wet seed surface priming—and on seeds submerged in water—EHDP-immersed seed priming. These techniques were applied to green oak leaf lettuce (*Lactuca sativa* L.) seeds. The results show that EHDP-immersed seed priming significantly enhances germination rate (98.3%, compared to a control of ∼80%), germination uniformity (∼5 hrs compared to a control of ∼15 hrs), and the Mean Germination Time (MGT) (∼1.87 days compared to a control of ∼2.5 days). The presented method exploits three primary plasma formation regions, each generating distinct reactive oxygen and nitrogen species (RONS) and ions that interact differently with the seeds. RONS, particularly hydrogen peroxide (H_2O_2), nitrate (NO_3^-), and nitrite (NO_2^-), play crucial roles in germination, vigor, nutrient uptake, and hormonal regulation, thereby effectively breaking seed dormancy. Potential plasma treatment damages were addressed, revealing no significant variations in plant height, root length, leaf diameter, leaf thickness, or leaf numbers between control and plasma-primed groups, affirming EHDP plasma priming's safety. This study underscores the EHDP plasma priming's potential to enhance seed germination and early plant growth, while also reducing contamination risks without negatively impacting plant health and development, indicating its transformative potential for the agricultural industry.

INDEX TERMS Atmospheric-pressure plasmas, arc discharges, electrohydraulic discharge plasma, gas-liquid interfacial plasma, seed priming.

I. INTRODUCTION

Seed priming, a technique used to improve germination and seedling growth in various crops before planting, accelerates the germination process by softening the seed coat and promoting pre-germinative metabolism [1], [2], [3]. Common methods, such as hydropriming and osmopriming, involve treating seeds with water or specific solutions such as potassium nitrate (KNO_3) and polyethylene glycol (PEG) for typically ∼24 to ∼48 hours [2], [3], [4]. Despite their effectiveness, these techniques have limitations, such as the potential for over-soaking or high-temperature exposure leading to low germination rates and impaired seedling growth [2], [3]. Moreover, soaking seeds can introduce contamination risks from microorganisms or pathogens [3], and the need for constant monitoring and water replacement makes these techniques laborious and time-consuming. Some seed priming approaches involve using chemicals, which can be costly and detrimental to the environment [3].

Recently, cold plasma technology has gained significant attention as an innovative and environmentally friendly method for enhancing seed germination and plant growth [1], [5]. By promoting water absorption and nutrient uptake, cold plasma can effectively improve plant health and yield [6], [7], [8], [9]. However, traditional nonthermal plasma technologies for seed treatment are frequently hampered by

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the need for specific inert gases and vacuum equipment, limiting their accessibility [6], [9], [10], [11]. On the other hand, atmospheric nonthermal plasma has emerged as a more practical alternative, demonstrating its ability to successfully disinfect seeds [6], [12], [13], [14] and improve their resistance to diseases [12] without the need of a vacuum system, making it a valuable asset to the agricultural industry offering cost-effective and eco-friendly solutions for seed treatment. Recently, gas-liquid interface plasma (GLIP) has been investigated as a promising technique capable of generating reactive species in both gaseous and liquid states [11], [13], [14], [15], [16]. GLIP has shown great potential for efficiently eliminating harmful contaminants and modifying material properties in various applications [11], [13], [14], [15], including water treatment [11], [13], [16], food sterilization [12], and material synthesis [17]. However, effectively exploiting the benefits of GLIP, which generates a wide variety of short-lived reactive species (with a half-life ranging from nanoseconds to seconds) and long-lived species (with a half-life ranging from minutes to months), presents a formidable challenge. Previously, we successfully demonstrated that electrohydraulic discharge plasma (EHDP) induced gas-liquid interface plasma could effectively deteriorate *Alternaria brassicicola*, a contaminant found in Chinese kale seeds [14]. Due to the abundance of reactive species and ions, we anticipated that the highly localized GLIP could also improve seed metabolism when used for priming. However, using EHDP methods to induce plasma at the gas-liquid interface for seed priming remains relatively unexplored.

Herein, we investigate the use of EHDP-induced GLIP via a gliding arc configuration for green oak leaf lettuce (*Lactuca sativa* L.) seed priming to gain a deeper understanding of this innovative technology and its potential applications in sustainable agriculture. We performed two distinct EHDP approaches to achieve GLIP interaction on the seeds. In the first method, EHDP was applied directly to the pre-soaked seeds (Figure 1a). In the second approach, the EHDP was applied to immersed seeds (Figure 1b). We then performed a germination test to investigate the effect of EHDP on seed germination parameters for 7-14 days after treatment, followed by a study of plant growth parameters during standard hydroponic planting for ~42 days. The growth parameters of plasma-treated seeds were compared to the growth parameters of conventional priming methods to obtain a better understanding of the behavior, underlying mechanisms, and limitations of EHDP priming. Our work provides valuable insights for farmers and researchers looking to optimize nonthermal plasma for seed priming while also enhancing the scalability of EHDP for large-scale seed priming and minimizing its cost and impact on the environment.

II. RESULTS AND DISCUSSION

Figures 1a and 1b demonstrate two distinct EHDP techniques for producing GLIP interaction on the seeds. In the first scenario (Figure 1a), we applied EHDP to wet seeds, a process we refer to as EHDP-wet seed surface priming. In the second condition, EHDP was applied to immersed seeds as EHDP-immersed seed priming (Figure 1b). When a high-voltage pulse is applied between two diverging electrodes, it causes a gliding arc discharge, forming a thermal plasma with extremely high temperatures reaching tens of thousands of degrees Kelvin [11], [18], [19]. However, by introducing a high-velocity airflow of approximately 10 L/min through a gap between these electrodes, we can create an atmospheric nonthermal plasma rich in reactive species, ions, radicals, and excited molecules (see material and method for nonthermal plasma setup and configuration).

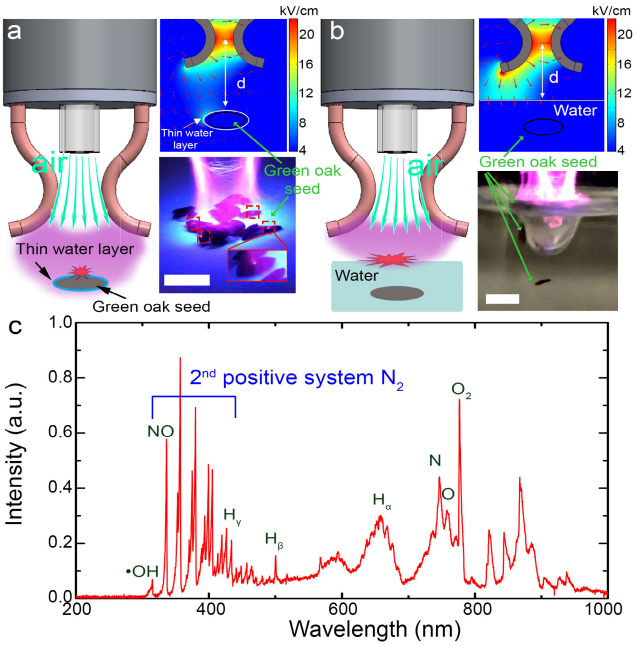

FIGURE 1. Electrohydraulic discharge plasma (EHDP) induced gas-liquid interface for seed priming. Schematic representations of (a) EHDP-wet seed surface priming and (b) EHDP-immersed seed priming. In the EHDP-wet seed surface priming process, a thin water layer coating the seed permits electrical discharge on the water surface, resulting in nanoscale heating spots (highlighted in inset-red-dashed boxes) on the seed. The top-inset shows the initial electric field (Unit: kV/cm) distribution, while the bottom-inset shows the optical image of each EHDP treatment (scale bar ~0.5 cm). In the EHDP-immersed seed priming process, seeds are submerged in water, and the electrical discharge is directly introduced into the water. Both methods generate a high-density gas-liquid interface plasma that leads to different interactions with the seeds. (c) Optical emission spectra (OES) obtained during the EHDP process confirm the presence of various reactive gaseous species at specific wavelengths: atomic and molecular oxygen (O and O_2), atomic and molecular nitrogen (N and N_2), hydroxyl radicals (OH), nitric oxide (NO), and ozone (O_3), along with positive and negative ions generated under air discharge.

In EHDP-wet seed surface priming (Figure 1a), the thin water layer that coats the seed serves as a virtual ground, permitting an electrical discharge on the water surface. As a result, the EHDP generates nanoscale heating spots (bright spots in Figure 1a-bottom inset-red dashed boxes) on the seed, indicating the presence of a high-density gas-liquid interface plasma with ions, electrons, and reactive species

such as OH, H_2O_2, ONOO, and NO [11], [13], [14], [15], [16], [20], similar to the gas-liquid interface region shown in Figure 3a. We therefore anticipate that short-lived Reactive Oxygen and Nitrogen Species (RONS) such as the hydroxyl radical (OH) and singlet oxygen ($^1O_2$) (half-life in the microsecond to nanosecond range), Nitric oxide radical (NO) with a half-life of 1 ms and peroxynitrite (ONOO-) with a half-life of 1 ms will interact strongly with the seed surface [13], [14], [15], [16]. Nevertheless, the duration of the EHDP-induced GLIP treatment for wet seeds is limited to 2 minutes. As the seed completely dries, the EHDP process will transition to typical gas-phase plasma as shown in the other area (Figure 1a-bottom inset), similar to previous reports [14], [21]. Therefore, the total treatment time of EHDP-wet seed surface priming is approximately 2 minutes. Figure 1a-inset presents the initial electric field distribution for EHDP-wet surface priming. A high electric field (∼30 kV/cm) develops across the diverging electrodes with a 5 mm gap, sufficiently causing an electrical arc between them [21], [22]. Introducing air into the arc region, however, not only transports plasma components, such as electrons and reactive gaseous species, to the treated samples, but also replaces the breakdown gas, resulting in continuous non-thermal plasma generation.

For EHDP-immersed seed priming (Figure 1b), the initial electric field distribution will be concentrated around the diverging electrodes and the electrode edge (Figure 1b-top inset). Similar to EHDP-wet seed surface priming, air will transport plasma components like electrons and reactive gaseous species to water. In addition, the electric field distribution confirms that a high electric field develops between the electrode edge and the water surface, leading to direct discharge into the water and subsequently resulting in the generation of EHDP, GLIP, and plasma-activated water (PAW). Figure 1b-inset depicts the optical image of EHDP-immersed seed priming. Although we anticipate RONS generation and interactions with the seed to be dominated by short-lived species in the GLIP region (Figure 3a-GLIP region) and long-lived species in the PAW region (Figure 3a-PAW region), the EHDP-immersed seed priming method does not result in localized nanoscale heating spots. Consequently, we anticipate a stronger interaction between seed and long-lived RONS in the liquid phase during priming. The total treatment time of EHDP induced GLIP and PAW (Figure 1b) is ∼1 min and the seeds were subsequently primed in this PAW for 12 hours before they underwent germination tests. Thus, the immersed seeds will be exposed to stable, long-lived species in the plasma-activated water (PAW) region, including hydrogen peroxide (H_2O_2) and nitrate (NO_3), each of which has a half-life extending to years [13], [20] (Figure 3a- PAW phase).

Figure 3c illustrates the optical emission spectra (OES) obtained during the EHDP process, confirming the presence of various reactive gaseous species known to play an essential part in plasma-induced seed germination enhancement. The reactive species include atomic and molecular oxygen (O and O_2, at wavelengths ∼777 nm and ∼760 nm, respectively), atomic and molecular nitrogen (N and N2, at wavelengths ∼746 nm and ∼337 nm, respectively), hydroxyl radicals (OH, at a wavelength around ∼309 nm), nitric oxide (NO, at a wavelength around ∼237 nm), and ozone (O_3, at a wavelength around ∼254 nm), along with positive and negative ions generated under air discharge [13], [20]. However, gas-phase and liquid-phase plasma can interact with seeds in different ways [13], [16], [20]. To elucidate the effect of EHDP on seed priming, we conducted a germination test on seeds treated with EHDP-wet seed surface priming and EHDP-immersed seed priming, comparing the germination results to those from osmopriming and hydropriming methods (see material and method for each priming procedure).

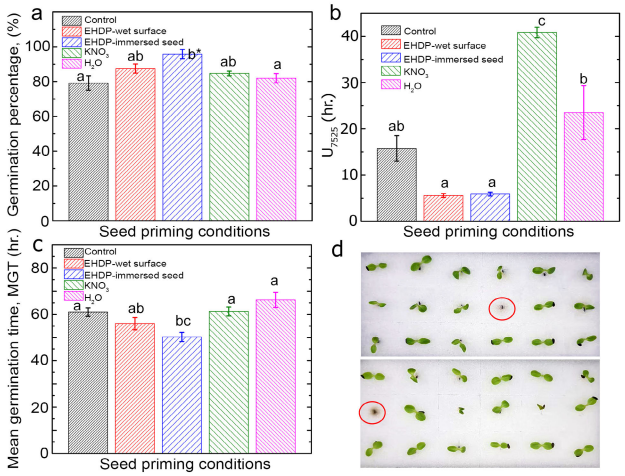

FIGURE 2. Comparative germination parameters for green oak seeds treated with different priming methods. (a) Average germination percentage of control seeds (black-bar), seeds treated with EHDP on wet seed surface (EHDP-wet seed surface priming, red-bar), seeds treated with EHDP on immersed seeds (EHDP-immersed seed priming, blue-bar), seeds treated with osmopriming (0.5% KNO_3, green bar), and seeds treated with hydropriming (H_2O, purple bar). (b) The uniformity of germination, denoted by U_{7525} values, across the different priming methods. Lower U_{7525} values indicate a greater uniformity of germination. (c) Mean germination time (MGT) for each priming method. Lower MGT values represent faster germination. (d) Representative image of green oak seeds after 7 days of germination. The red circle highlights an unsuccessfully germinated seed. The data represent the mean of four replicates ± 2 standard error of the mean with different lower-case letters indicating a significant difference ($p < 0.05$).

Figure 2 demonstrates the effects of different priming methods on the germination parameters of green oak seeds on the 7th day after sowing. There are five types of seed samples, control seeds (nontreated, black-bar), the EHDP-wet seed surface priming (red-bar), the EHDP-immersed seed priming, (blue-bar), osmopriming seeds (0.5% KNO_3, green bar), and hydropriming (H_2O, purple bar) (see material and method for priming process). The results indicate that EHDP-immersed priming seeds (Figure 2a-blue bar) have the highest average germination percentage (95%) with a statistically significant difference from control (80%, Figure 2a-black bar) and hydropriming (82%, Figure 2a-purple bar). Although the EHDP-wet seed surface priming also exhibits an average

germination percentage of ∼88% (Figure 2a, red bar), there is no significant difference between this treatment and the other treatments. Nonetheless, the standard error of the mean suggests that osmopriming with KNO_3 resulted in the most consistent germination percentage (85%, as shown in Figure 2a-green bar). This indicates that priming methods using liquid-based solutions offer more uniform seed interaction in terms of volume distribution. Even though the average germination percentages of hydropriming and osmopriming are higher than the control, these treatments did not have a statistically significant effect when compared to the control, our results indicate that the green oak seeds used in our experiment were already viable and of high quality.

Figure 2d shows representative data of green oak seeds after 7 days of germination. Figure 2d highlights (in a red circle) a seed that did not successfully germinate, while the other seeds are healthy seedlings. Our findings provide further support that the EHDP treatment on immersed seeds shows promise as a priming method that can improve the germination percentage of seeds. This finding is consistent with previous research which suggested that plasma-based seed priming methods are effective in improving seed germination and emergence due to the availability of ions, nutrients, and reactive species [6], [7], [20]. Consistent with previous research [23], the use of pure water in hydropriming alone may not be an effective priming procedure for increasing the germination percentage of green oak lettuce seeds. Using osmopriming solutions can be more effective than hydropriming at increasing the germination rate and seedling growth, but chemical substances are required, and they can leave chemical residues in the environment and samples [3].

In addition to the germination percentage, we also evaluated other important germination parameters that provide insight into the effectiveness of different priming methods. Specifically, we analyzed the uniformity of germination known as U7525 representing the time interval between 75% and 25% of viable seeds to germinate and the mean germination time (MGT) [24] of the different priming methods. The smaller values indicate greater uniformity of germination. Figure 2b, demonstrates that both EHDP treatments showed significantly lower U7525 values (∼5 hours) compared to the control group (∼15 hours), osmopriming (∼40 hours), and hydropriming (∼20 hours). These results indicate that employing plasma treatment for priming seeds can provide high uniformity of seed germination, which is consistent with previous studies that have demonstrated the positive effects of plasma treatment on seed germination and growth [4], [7]. On the other hand, the U7525 values for hydropriming and control samples were not significantly different, suggesting that hydropriming did not enhance the uniformity of germination. This finding is consistent with previous studies that have reported mixed results on the efficacy of hydropriming in improving seed germination [23]. Figure 2c displays the MGT for various priming methods. The results reveal that EHDP-immersed priming seeds had the shortest MGT at ∼50 hours, whereas other methods showed no significant difference in MGT at ∼60 hours. This study suggests that EHD-water plasma treatment can accelerate germination in green oak seeds, consistent with previous research on plasma treatment for seed priming [20]. Plasma treatment may boost the physiological and biochemical processes in seed germination, resulting in quicker, more uniform germination [7], [13]. In comparison, osmopriming and hydropriming methods did not exhibit a significant MGT difference compared to the control group. These findings imply that EHD-water plasma treatment could be a promising technique for improving seed germination speed and efficiency, with significant implications for crop production, as enhanced uniform germination can contribute to better crop yields and reduced losses.

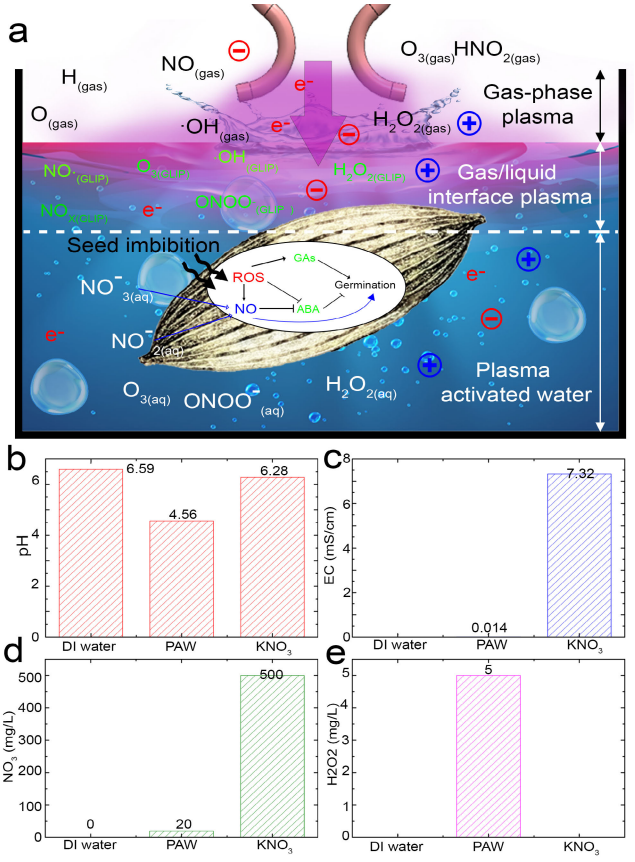

FIGURE 3. Underlying mechanism of EHDP-immersed seed priming and characterization of the resulting plasma-activated water (PAW). (a) Schematic illustrating the three primary plasma formation regions during EHDP-immersed seed priming: gas-phase plasma, gas-liquid interface plasma (GLIP), and liquid phase plasma (PAW). The schematic also displays a complex network of chemical reactions inside the seed induced by the reactive species in PAW, influencing seed germination, nutrient uptake, hormonal regulation, and metabolic pathway activation. (b) The pH comparison in PAW, deionized (DI) water, and 0.5% KNO_3 solution reveals the increased acidity of PAW due to various reactive oxygen and nitrogen species (RONS). (c-e) Differences in electrical conductivity, nitrate (NO_3^-) levels, and hydrogen peroxide (H_2O_2) levels in PAW, DI water, and a 0.5% KNO_3 solution. These physicochemical properties of PAW suggest its potential to contribute to faster, more consistent germination and healthier seedlings.

To understand the efficacy of EHDP-immersed seed priming, it is essential to explore its underlying mechanisms.

Figure 3a illustrates RONS production and seed interactions during EHDP-immersion seed priming, including the underlying mechanism. During treatment, immersed seeds continuously move to the water surface and then back beneath it due to the ∼10L/min air flowing into the water (Figure 1b). Consequently, during the EHDP-immersed seed priming process, there are three primary plasma formation regions with distinct interactions with the seeds. The first region consists of gas-phase plasma generated by a gliding arc discharge. The gas-phase plasma consists of UV light, electrons, ions, and reactive oxygen and nitrogen species (RONS) [13], [21]. In contrast to conventional plasma treatment, the gas-phase plasma components used in EHDP-immersed seed priming have no direct effect on the seeds. Instead, the gaseous plasma components determine the reactive species present in the secondary plasma region, referred to as the gas-liquid interface plasma (GLIP), and the third plasma phase, known as liquid phase plasma or plasma-activated water (PAW).

At the gas-liquid interface, air plasma-generated species include $NO_2\cdot$, $NO\cdot$, $OH\cdot$, and NO^+ ions, as well as hydroxyl radicals ($\cdot OH$) as short-lived reactive species formed in both gas and liquid phases that readily interact with the samples. However, because the slow transport across the gas-liquid interface limits the transfer of reactive species from the gas phase to the water phase, only a subset of active species in gaseous plasma, such as O_3, H_2O_2, and O_2^-, as relatively stable long-lived species, can penetrate the gas-water interface and exist in the liquid phase. Thus, the third phase (Figure 3a) is the liquid phase plasma which involves the transformation of water into plasma-activated water (PAW). In PAW, several reactive oxygen species (ROS) such as hydroxyl radicals ($\cdot OH$), singlet oxygen ($^1O_2$), superoxide anions (O_2^-), hydrogen peroxide, ozone, and reactive nitrogen species (RNS) such as nitric oxide ($NO\cdot$) and its derivatives formed in water, including nitrite (NO_2^-), nitrate (NO_3^-), nitrogen radicals, nitrous acid (HNO_2), nitric acid (HNO_3), and peroxynitrite ($ONOO^-$) can alter the pH, conductivity, and redox potential properties of PAW. Figure 3b-3e demonstrate the physiology of PAW after plasma treatment in the EHDP-immersed seed, including pH, electrical conductivity, NO_3, and H_2O_2, compared to those of deionized (DI) water used for hydropriming and 0.5% KNO_3 used for osmopriming. Figure 3b shows that the pH of PAW is ∼4.68, indicating more acidity, while the pH of DI water and KNO_3 is still in the neutral range ∼6.5. The generation of various RONS in the liquid upon air plasma treatment endows PAW with acid-base properties, as hydrogen ions are released in the aqueous solution, and the dissolution of NO_2^- and NO_3^- produces nitrate or nitrite acid in the liquid phase. The formation of RONS contributes to an increase in conductivity and a decrease in pH in the liquid phase. The change in electrical conductivity of the liquid is attributed to the presence of charged ions and species in PAW.

Beyond these characteristics, it is equally important to consider the role of RONS in the germination and vigor of submerged seeds treated with EHDP. RONS, including hydrogen peroxide (H_2O_2), nitrate (NO_3^-), and nitrite (NO_2^-), are produced in the Gas-Liquid Interfacial Plasma (GLIP) and Plasma Activated Water (PAW) regions. Although the conductivity and nitrate components of PAW significantly increase to ∼14 μS/cm and 20 mg/L, respectively, these values remain incomparable to the conductivity of KNO_3 used in osmopriming, which is ∼100 mS/cm and >500 mg/L. However, PAW is the only treatment containing reactive oxygen species such as H_2O_2, which is considered a long-lived species with antimicrobial properties. Regarding the germination parameter results (Figure 2), RONS generated in the GLIP, and PAW region may provide the positive effects of abscisic acid (ABA) on seed dormancy and serve as nutrient sources for germination [7], [20]. Nitrogen fertilization increases NO release, which breaks dormancy and promotes germination in various seeds [7], [13]. Similarly, nitrate and nitrite in PAW are assimilated by seedlings, providing essential nutrients to improve germination rates and seedling vigor. Figure 3a (inside the seed) demonstrates a complex network of chemical reactions involving reactive species that can influence seed germination, nutrient uptake, hormonal regulation, and metabolic pathway activation. For example, the presence of RONS, including NO, NO_2^-, and NO_3^-, in PAW promotes germination by decreasing ABA levels and increasing gibberellins (GA) levels, which enhance germination. H_2O_2 plays a vital role not only in the sterilization of the contaminated microorganism but also in breaking seed dormancy by stimulating enzymes that weaken the seed coat, allowing water penetration and initiating germination. Reactive oxygen species (ROS) and nitric oxide (NO) work synergistically in this process, potentially acting upstream of ABA [20]. Therefore, the EHDP-immersed priming is an exogenous nitrate treatment that can effectively reduce ABA levels and release seed dormancy, particularly in seeds with hard coats. Nitric oxide (NO) is thought to be a key signaling element in the nitrate response in seeds. Therefore, overall results indicated that the EHDP-immersed seed priming can contribute to faster, more consistent germination and healthier seedlings, ultimately promoting overall plant growth and development.

Consistent with previous research on using plasma for priming [25], our study demonstrates that EHDP can enhance the germination percentage and uniformity of germination, suggesting its potential use in the priming process. While the benefits of EHDP in enhancing germination and uniformity are clear, there have been concerns in the research community about possible damage to plant cells or tissues caused by plasma treatments [13]. Furthermore, while the positive impacts of EHDP on seed germination are evident, the question of how it affects subsequent plant growth remains. To address these concerns, we further investigated the effects of two plasma priming methods, EHDP-wet seed surface priming and EHDP-immersed seed priming, on the growth characteristics of green oak lettuce and compared them with a control group of lettuce plants.

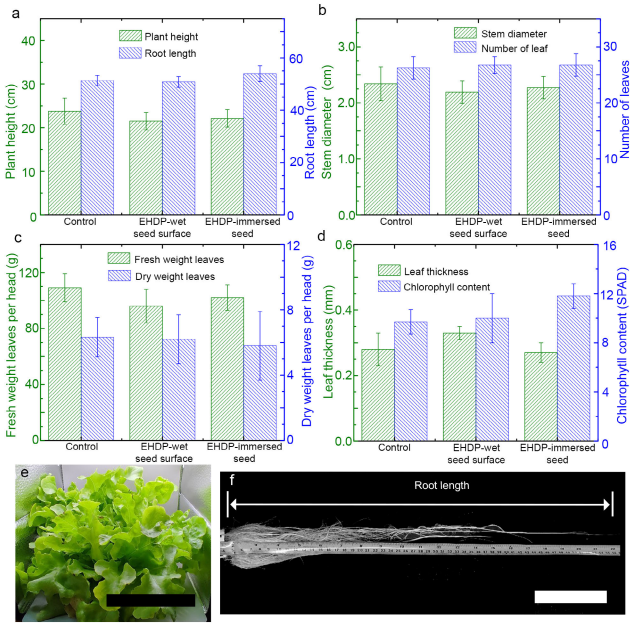

FIGURE 4. Growth characteristics of green oak lettuce following different priming methods. (a) Plant height and root length comparison among the control group, EHDP-wet seed surface priming group, and EHDP-immersed priming group, respectively. (b) Leaf diameter and number of leaves comparison among different priming methods. (c) Fresh weight and dry weight of leaves comparison among different priming methods. (d) Leaf thickness and chlorophyll content comparison among the control group and both plasma-primed groups. (e-f) Representative images showing the appearance and root length of green oak lettuce plants following each priming method (scale bar ∼10 cm). The data were accumulated from a minimum of four independently replicated trials (n≥4) and represent the mean ± 2 standard error with a confidence threshold set at $p < 0.05$.

After 14 days of seedling production, we transported the plants to the IoT controlled greenhouse and transplanted them into the same standard hydroponic greenhouse system under constant lighting conditions and closely controlled temperature, relative humidity, carbon dioxide, and irrigation [26], [27]. The plants were grown for a period of 35 days, after which they were harvested. Figures 4a to 4d illustrate the plant growth parameters of lettuce grown using different priming methods (4a- plant height and root length, 4b- leaf diameter, number of leaves, 4c-fresh weight and dry weight of leaves, 4d- leaf thickness and chlorophyll content). Our results indicate no significant differences in key growth parameters - plant height, root length, stem diameter, leaf thickness, and number of leaves - between the control group and both plasma-primed groups (Figures 4a, 4b and 4d). On average, lettuce plants had a height and root length of ∼23 cm and ∼50 cm respectively. The average stem diameter was ∼2.25 cm, average leaf thickness was ∼0.3 cm, and the average number of leaves per plant was ∼26. Although the chlorophyll content of the EHDP-immersed priming group was slightly higher than the other groups, this difference was not statistically significant (Figure 4b-4d) (see material and method). Figure 4e-4f show representative images of green oak leaves and the root length of green oak lettuce plants grown following each priming method. The results confirm that EHDP-wet seed surface priming and EHDP-immersed priming do not cause any harm to seedlings after planting in a hydroponic system. EHDP plasma priming can enhance seed germination and early plant growth without any negative impact on the overall health and development of the plant.

Our results are consistent with previous research and contribute to the existing literature on the effectiveness of plasma priming for the agricultural industry [13]. EHDP plasma priming could help improve seed germination, assist in decontamination during early plant growth stages [14], [16], and have no negative impact on the overall health and development of the plant. The added benefit of plasma sterilization could also reduce contamination risks and promote healthier plant growth [6], [14], leading to higher crop yields and better food security. However, our results are in line with previous research suggesting that growth characteristics in hydroponic systems are primarily determined by factors like primary fertilizer, light, pH, and CO_2 [26], [28]. Thus, further research is needed to fully understand the mechanisms underlying plasma priming and optimize the technique for using plasma-activated water as fertilizer to reduce the use of chemical fertilizers, promote a greener and more sustainable approach, and enhance crops.

III. CONCLUSION

In conclusion, our study contributes significantly to the understanding of the EHDP seed priming process, demonstrating its potential to enhance seed germination, uniformity, and subsequent plant growth. Our findings illustrate the transformative role EHDP-immersed seed priming plays in the alteration of seed dormancy and stimulation of seed germination. Reactive Oxygen and Nitrogen Species (RONS), generated during the EHDP-immersed seed priming process, play an essential role in breaking dormancy, promoting seed germination, and may be the driving force behind the enhanced seed germination and uniform growth we observed. After closely monitoring plant growth characteristics post-treatment through various metrics such as plant height, root length, and leaf thickness, we observed no discernible negative effects on the plants' overall health and development. The increase in germination and seedling growth uniformity suggests that the EHDP-immersed seed priming can potentially optimize crop yields. However, while this study offers promising insights, it is crucial to note that multiple factors, such as primary fertilizer, light, pH, and CO_2, likely also influence growth characteristics in hydroponic systems. Further research is required to enhance our understanding of the underlying mechanisms of plasma priming and to optimize the technique. This should include investigating the use of plasma-activated water as a sustainable alternative to chemical fertilizers.

IV. MATERIAL AND METHOD

A. GLIDING ARC DISCHARGE PLASMA

The gliding arc plasma generator used in the experiment was equipped with two electrodes, distanced ∼5 mm apart, and

was supplied with a voltage of 10 kVp, a frequency of 10 kHz, and an airflow of 10 L/min. A high-voltage pulse was applied between these diverging electrodes, with one electrode linked to a positive high voltage of up to 10 kVp, while the other was connected to the ground. The distance (d) between the surface of the Green Oak seed and the electrode tip of the plasma was maintained at 10 mm.

B. SEED MATERIAL

The commercial green oak leaf lettuce (*Lactuca sativa*) seeds used in this study were purchased and formally identified from CHUA YONG SENG SEED CO., LTD., Thailand (Lot No. 160130). We selected uniformly sized and shaped seeds for the sake of experimentation consistency. Prior to the initiation of seed priming, these seeds were stored in a seedling box at a controlled temperature of 18°C and relative humidity of 42%.

C. SEED PRIMING METHODS

In the EHDP-wet method, 100 green oak seeds were briefly immersed in 100 mL of deionized water for one minute and then treated with gliding arc plasma for two minutes. Conversely, the EHDP-immersed method involved immersing the seeds in 100 mL of deionized water and exposing them to gliding arc plasma for ∼1 min, creating plasma-activated water (PAW). The seeds were then primed in this PAW for 12 hours before undergoing germination tests. Traditional hydropriming and osmopriming methods were also used for comparison [2], [3], [4], [23]. Hydropriming required a 12-hour immersion of seeds in deionized water in a light-controlled room, while osmopriming involved soaking the seeds in a low-electrical conductivity solution of 0.5% KNO_3, stored similarly for around 12 hours. All chemicals used in this study were analytical grade, and only deionized water was employed.

D. MEASUREMENT OF LEAF CHLOROPHYLL CONCENTRATIONS

The SAPD 502 PLUS Chlorophyll meter was employed to determine the relative amount of chlorophyll present by measuring the absorbance of the leaf in two wavelength regions.

E. GERMINATION TEST

A germination experiment was implemented in a greenhouse, managed by an Internet of Things (IoT) system, as portrayed in Fig. 3. The study used five distinct seed groups, each subjected to different priming methods: EHDP-wet seed surface priming, EHDP-immersed seed priming, hydropriming, osmopriming, and a control group that did not undergo any priming. To ensure reliability, the germination test was replicated four times. Each test involved the placement of 100 Green Oak seeds into a seedling box (dimensions ∼10.2 × ∼21 × ∼5.6 cm^3) filled with polyurethane foam moistened with reverse osmosis (RO) water. A LED light source (TS1000) of dimensions ∼38.2 cm × ∼33.6 cm × ∼54.5 cm provided maximum illumination, facilitating optimal germination conditions. The Green Oak seeds were subjected to a range of photosynthetic photon flux densities (PPFDs) between ∼120 and ∼270 $\mu mol/m^2/s$, favorable for germination [29]. The lighting protocol comprised 8 hours of LED light exposure followed by a 16-hour rest period, cyclically repeated for seven days. This ensured optimal and consistent light conditions for germination. Germination generally commenced on the fourth day post-planting and by the seventh day, all viable seeds had germinated. Monitoring the germination over this period allowed for assessing the effectiveness of the varied seed priming techniques on Green Oak seedling germination.

F. OPTICAL EMISSION SPECTROSCOPY (OES)

In the Optical Emission Spectroscopy (OES) analysis, a fiber optic cable was connected to the Charge-Coupled Device (CCD) (Thorlabs CCS200 unit), and its other end was located close to the gliding arc discharge plasma region. The system processed photon signals in the 200-1000 nm wavelength range with a spectral resolution of less than 2 nm and a full-width half-maximum of 633 nm.

G. FINITE ELEMENT METHOD (FEM) SIMULATION

The electrostatic field distribution during the initial operation of the gliding arc plasma was analyzed using a finite element solution conducted through COMSOL MULTIPHYSICS.

H. STATISTICAL ANALYSIS

For every treatment protocol, data were accumulated from a minimum of four independently replicated trials ($n \geq 4$). The results for each experiment are presented as the mean $\pm$ 2 standard error of the mean. Statistical significance was assessed using a one-way analysis of variance (ANOVA) with a confidence threshold set at $P < 0.05$.

CONFLICTS OF INTEREST

The authors declare no competing financial interest.

REFERENCES

[1] L'. Holubová, S. Kyzek, I. Ďurovcová, J. Fabová, E. Horváthová, A. Ševčovičová, and E. Gálová, "Non-thermal plasma—A new green priming agent for plants?" *Int. J. Mol. Sci.*, vol. 21, no. 24, p. 9466, Dec. 2020. [Online]. Available: https://www.mdpi.com/1422-0067/21/24/9466

[2] S. Paparella, S. S. Araújo, G. Rossi, M. Wijayasinghe, D. Carbonera, and A. Balestrazzi, "Seed priming: State of the art and new perspectives," *Plant Cell Rep.*, vol. 34, no. 8, pp. 1281–1293, Aug. 2015.

[3] K. Sivasubramaniam, R. Geetha, S. Kandasamy, K. Raja, A. Sripunitha, and R. Selvarani, "Seed priming: Triumphs and tribulations," *Madras Agric. J.*, vol. 98, pp. 197–209, Sep. 2011.

[4] S. I. Shim, J.-C. Moon, C. S. Jang, P. Raymer, and W. Kim, "Effect of potassium nitrate priming on seed germination of seashore paspalum," *HortScience*, vol. 43, no. 7, pp. 2259–2262, Dec. 2008.

[5] P. Attri, K. Ishikawa, T. Okumura, K. Koga, and M. Shiratani, "Plasma agriculture from laboratory to farm: A review," *Processes*, vol. 8, no. 8, p. 1002, Aug. 2020. [Online]. Available: https://www.mdpi.com/2227-9717/8/8/1002

[6] N. Khamsen, D. Onwimol, N. Teerakawanich, S. Dechanupaprittha, W. Kanokbannakorn, K. Hongesombut, and S. Srisonphan, "Rice (*Oryza sativa* L.) seed sterilization and germination enhancement via atmospheric hybrid nonthermal discharge plasma," *ACS Appl. Mater. Interfaces*, vol. 8, no. 30, pp. 19268–19275, Aug. 2016, doi: 10.1021/acsami.6b04555.

[7] P. Ranieri, N. Sponsel, J. Kizer, M. Rojas-Pierce, R. Hernández, L. Gatiboni, A. Grunden, and K. Stapelmann, "Plasma agriculture: Review from the perspective of the plant and its ecosystem," *Plasma Process. Polym.*, vol. 18, no. 1, 2021, Art. no. 2000162, doi: 10.1002/ppap.202000162.

[8] T. Rongsangchaicharean, K. Ruangwong, D. Onwimol, N. Tephiruk, S. Suwannarat, and S. Srisonphan, "Effect of dielectric barrier discharge plasma on rice (*Oryza sativa* L.) seed hydration and hygroscopicity," *J. Phys. D, Appl. Phys.*, vol. 55, no. 36, Sep. 2022, Art. no. 365201, doi: 10.1088/1361-6463/ac791d.

[9] L. Ling, J. Jiafeng, L. Jiangang, S. Minchong, H. Xin, S. Hanliang, and D. Yuanhua, "Effects of cold plasma treatment on seed germination and seedling growth of soybean," *Sci. Rep.*, vol. 4, no. 1, p. 5859, Jul. 2014, doi: 10.1038/srep05859.

[10] N. N. Misra, O. Schlüter, and P. J. Cullen, *Cold Plasma in Food and Agriculture*. San Diego, CA, USA: Academic Press, 2016, pp. 1–16.

[11] B. Jiang, J. Zheng, S. Qiu, M. Wu, Q. Zhang, Z. Yan, and Q. Xue, "Review on electrical discharge plasma technology for wastewater remediation," *Chem. Eng. J.*, vol. 236, pp. 348–368, Jan. 2014, doi: 10.1016/j.cej.2013.09.090.

[12] N. N. Misra, B. Yadav, M. S. Roopesh, and C. Jo, "Cold plasma for effective fungal and mycotoxin control in foods: Mechanisms, inactivation effects, and applications," *Comprehensive Rev. Food Sci. Food Saf.*, vol. 18, no. 1, pp. 106–120, Jan. 2019, doi: 10.1111/1541-4337.12398.

[13] Y. Gao, K. Francis, and X. Zhang, "Review on formation of cold plasma activated water (PAW) and the applications in food and agriculture," *Food Res. Int.*, vol. 157, Jul. 2022, Art. no. 111246, doi: 10.1016/j.foodres.2022.111246.

[14] S. Suwannarat, C. Thammaniphit, and S. Srisonphan, "Electrohydraulic streamer discharge plasma-enhanced *Alternaria brassicicola* disinfection in seed sterilization," *ACS Appl. Mater. Interfaces*, vol. 13, no. 37, pp. 43975–43983, Sep. 2021, doi: 10.1021/acsami.1c10771.

[15] T. Kaneko, S. Sasaki, K. Takashima, and M. Kanzaki, "Gas-liquid interfacial plasmas producing reactive species for cell membrane permeabilization," *J. Clin. Biochem. Nutrition*, vol. 60, no. 1, pp. 3–11, 2017, doi: 10.3164/jcbn.16-73.

[16] B. R. Locke, M. Sato, P. Sunka, M. R. Hoffmann, and J.-S. Chang, "Electrohydraulic discharge and nonthermal plasma for water treatment," *Ind. Eng. Chem. Res.*, vol. 45, no. 3, pp. 882–905, Feb. 2006, doi: 10.1021/ie050981u.

[17] L. Di, J. Zhang, X. Zhang, H. Wang, H. Li, Y. Li, and D. Bu, "Cold plasma treatment of catalytic materials: A review," *J. Phys. D, Appl. Phys.*, vol. 54, no. 33, Aug. 2021, Art. no. 333001, doi: 10.1088/1361-6463/ac0269.

[18] K. Knoerzer, A. B. Murphy, M. Fresewinkel, P. Sanguansri, and J. Coventry, "Evaluation of methods for determining food surface temperature in the presence of low-pressure cool plasma," *Innov. Food Sci. Emerg. Technol.*, vol. 15, pp. 23–30, Jul. 2012, doi: 10.1016/j.ifset.2012.02.008.

[19] A. C. Borges, K. G. Kostov, R. S. Pessoa, G. M. A. de Abreu, G. D. M. G. Lima, L. W. Figueira, and C. Y. Koga-Ito, "Applications of cold atmospheric pressure plasma in dentistry," *Appl. Sci.*, vol. 11, no. 5, p. 1975, Feb. 2021. [Online]. Available: https://www.mdpi.com/2076-3417/11/5/1975

[20] R. Zhou, J. Li, R. Zhou, X. Zhang, and S. Yang, "Atmospheric-pressure plasma treated water for seed germination and seedling growth of mung bean and its sterilization effect on mung bean sprouts," *Innov. Food Sci. Emerg. Technol.*, vol. 53, pp. 36–44, May 2019, doi: 10.1016/j.ifset.2018.08.006.

[21] S. Srisonphan and V. Kasemsuwan, "Field electron emission enhanced streamer cold plasma interaction on seed surface wettability," *Surf. Interfaces*, vol. 22, Feb. 2021, Art. no. 100877, doi: 10.1016/j.surfin.2020.100877.

[22] S. Li, S. Yu, and Y. Feng, "Progress in and prospects for electrical insulating materials," *High Voltage*, vol. 1, no. 3, pp. 122–129, Oct. 2016, doi: 10.1049/hve.2016.0034.

[23] A. Damalas, D. Koutroubas, and Fotiadis, "Hydro-priming effects on seed germination and field performance of faba bean in spring sowing," *Agriculture*, vol. 9, no. 9, p. 201, Sep. 2019. [Online]. Available: https://www.mdpi.com/2077-0472/9/9/201

[24] R. V. L. Joosen, J. Kodde, L. A. J. Willems, W. Ligterink, L. H. W. van der Plas, and H. W. M. Hilhorst, "Germinator: A software package for high-throughput scoring and curve fitting of arabidopsis seed germination," *Plant J.*, vol. 62, no. 1, pp. 148–159, Apr. 2010, doi: 10.1111/j.1365-313x.2009.04116.x.

[25] A. Shelar, A. V. Singh, P. Dietrich, R. S. Maharjan, A. Thissen, P. N. Didwal, M. Shinde, P. Laux, A. Luch, V. Mathe, T. Jahnke, M. Chaskar, and R. Patil, "Emerging cold plasma treatment and machine learning prospects for seed priming: A step towards sustainable food production," *RSC Adv.*, vol. 12, no. 17, pp. 10467–10488, 2022, doi: 10.1039/d2ra00809b.

[26] J.-S. Lee, D. Chandra, and J. Son, "Growth, physicochemical, nutritional, and postharvest qualities of leaf lettuce (*Lactuca sativa* L.) as affected by cultivar and amount of applied nutrient solution," *Horticulturae*, vol. 8, no. 5, p. 436, May 2022. [Online]. Available: https://www.mdpi.com/2311-7524/8/5/436

[27] A. Sharma, C. Manpoong, V. S. Devadas, B. D. Kartha, H. Pandey, and M. Wangsu, "Crop hydroponics, phyto-hydroponics, crop production, and factors affecting soilless culture," *ACS Agricult. Sci. Technol.*, vol. 2, no. 6, pp. 1134–1150, Dec. 2022, doi: 10.1021/acsagscitech.2c00243.

[28] B. M. Mampholo, M. Maboko, P. Soundy, and D. Sivakumar, "Postharvest responses of hydroponically grown lettuce varieties to nitrogen application rate," *J. Integrative Agricult.*, vol. 18, no. 10, pp. 2272–2283, Oct. 2019, doi: 10.1016/S2095-3119(19)62664-X.

[29] N. Kelly, D. Choe, Q. Meng, and E. S. Runkle, "Promotion of lettuce growth under an increasing daily light integral depends on the combination of the photosynthetic photon flux density and photoperiod," *Scientia Horticulturae*, vol. 272, Oct. 2020, Art. no. 109565, doi: 10.1016/j.scienta.2020.109565.

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