

Compact solar-powered plasma water generator: enhanced germination of aged seed with the corona dielectric barrier discharger

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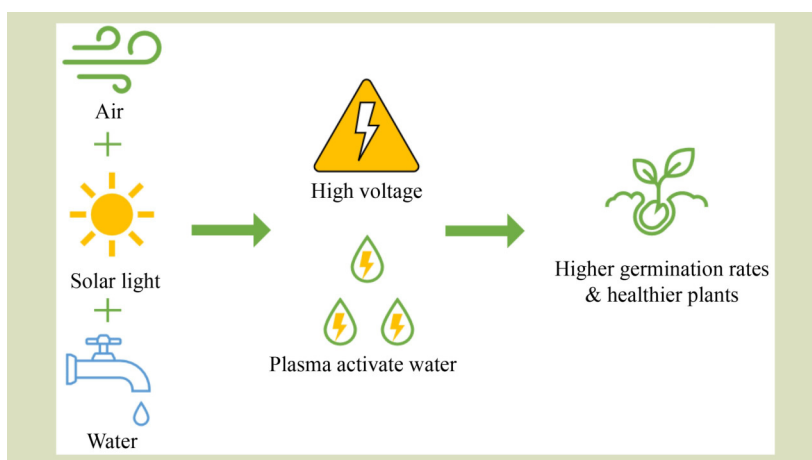
KEYWORDS

Non-thermal plasma, plant growth, reactor design, seed germination

HIGHLIGHTS

- Developed a novel solar-powered corona dielectric barrier discharge (cDBD) microreactor for sustainable agriculture.
- cDBD microreactor lowers pH and elevates oxidation-reduction potential, nitrite, and nitrate concentrations in plasma-activated water (PAW).
- PAW treatment doubled spinach seedling growth and increased germination rates by up to 135%.
- PAW modulates germination-related hormones to enhance aged-seed rejuvenation and growth.

GRAPHICAL ABSTRACT



ABSTRACT

Seed aging adversely affects agricultural productivity by reducing germination rates and seedling vigor, leading to significant costs for seed banks and companies due to the need for frequent seed renewals. This study demonstrated the use of plasma-activated water (PAW), generated by a solar-powered corona dielectric barrier discharger, to enhance germination rates of spinach seeds that had been stored at 4 °C for 23 years. Treating seeds with PAW at 17 kV for 15 min improved germination (by 135%) and seedling growth compared to untreated seeds. Through detailed analysis, beneficial PAW properties for seed development were identified, and a molecular mechanism for this rejuvenation is proposed. The solar-powered microreactor used in this study is considered to represent a significant advancement in seed treatment technology, offering a sustainable solution to meet growing food demands while addressing environmental and resource sustainability challenges.

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1 Introduction

Seed germination is of the utmost significance for seed banks and breeding institutions. It serves as a vital indicator of seed health and viability within their collections, ensuring the preservation of genetic diversity among plant species. Regular assessments of the germination process are necessary to ensure the maintenance of genetic integrity and to build a robust repository that accurately reflects the original diversity of these plant species. Breeding institutions rely heavily on successful germination to develop superior crop cultivars, as it allows breeders to select and advance seeds with desired genetic traits, ensuring these traits are expressed in future plant generations^[1]. In addition, the germination process is also a key factor in adaptive breeding for climate resilience^[2]. Both seed banks and breeding institutions prioritize seeds with higher germination rates, as these are often more adaptable to environmental stresses. This selection is important for developing crop cultivars that are resilient to changing climatic conditions and enhancing agricultural sustainability in diverse ecological settings^[3]. Lastly, in seed banks dedicated to endangered species, the germination success of stored seeds is pivotal for both conservation and species restoration programs^[4]. The capacity to achieve germination in these rare genotypes is essential for maintaining genetic diversity and is integral to reintroducing these species in their native habitats. In summary, the successful germination of seeds during storage is pivotal for genetic conservation, breeding advancements, climate resilience and restoration of endangered plant species, and thus it is indispensable for enhancing the long-term sustainability and adaptability of seed banks and breeding institutions.

Seed longevity varies significantly between species. While some seeds retain their vitality for centuries, others deteriorate within a few years. Despite optimal storage conditions designed to prolong germplasm viability, seed vitality inevitably declines over time. This decrease in germination is attributed to various factors. As seeds age, they suffer from viability loss, degradation of genetic material, moisture content fluctuations and sometimes less-than-ideal storage environments. The physical and metabolic changes in seeds, coupled with the buildup of germination-inhibiting substances, also lead to lower germination rates^[5]. Additionally, factors such as respiration, susceptibility to diseases, oxidative stress and alterations in the seed coat further diminish the germination potential of aging seeds^[6]. Therefore, developing methods to rejuvenate aged seeds and enhance their germination rates is not only about preserving genetic diversity, it is also important for maintaining a resilient and diverse germplasm repository,

which is vital for fostering sustainable agriculture and ensuring food security amidst evolving environmental challenges.

Various methods, such as gamma radiation^[7], ultrasound^[8], cold stratification^[9], scarification^[10], hormonal treatments^[11], biostimulants^[12], environmental control^[13], and salt solution pretreatment^[14], can be used to enhance seed germination. However, these techniques often face limitations like high labor and cost, the need for specific conditions or inconsistent results across different seed types. These constraints make them less universally applicable in agriculture. Recent research has highlighted the potential of non-thermal plasma (NTP)-treated water in significantly enhancing seed germination rates, and boosting overall health and vigor of seedlings. However, the implementation of NTP technology also presents some limitations, particularly regarding energy consumption and equipment costs. This high energy requirement poses challenges to its widespread application, especially in settings with limited resources. Also, while plasma-activated water (PAW) has shown promise in general seed germination, there is a notable gap in research specifically focusing on its effects on improving the germination rates of aged seeds.

Spinach is not only an important economic vegetable crop but also a valuable source of nutrients and health-promoting compounds, making it a beneficial part of the human diet. In 2022, the USA produced 428 kt of spinach^[15], maintaining its position as the second-largest spinach producer globally, underlining its significant to both the agricultural economy and public health. However, the rapid aging of spinach seed, with seed viability ranging from 2 to 5 years, poses challenges for seed storage and longevity. Old seeds are likely to produce less vigorous plants, with both the germination rate and plant vigor declining. Additionally, plants that reach maturity can be less productive and produce less seed, leading to significant economic losses.

To address these challenges, this study used a low-cost plasma generation device as outlined previously^[16], but powered with a solar cell. In this study, various parameters of the PAW under different conditions were measured, including pH, oxidation-reduction potential (ORP), and nitrite and nitrate concentrations. Additionally, the applied voltage and plasma treatment duration were optimized to achieve the highest seed germination rate. Also, the detailed mechanisms behind the generation of reactive species in PAW was considered in order to suggest how these influence germination at the molecular level. This analysis revealed the operational efficiency of the system and increased the understanding of seed germination enhancement processes.

2 Materials and methods

2.1 Experimental setup

A schematic of the NTP discharger is given in Fig. 1. The experimental reactor was assembled using a 20-mL glass vial as the main body, with an external diameter of 28 mm and a working volume of 12 mL. Copper wire (1 mm diameter) was used as the high voltage electrode and copper tape, 0.1 mm in thickness and 0.5 inches in length as the ground electrode being wound around the exterior of the glass vial. The gap facilitating electrical discharge between these electrodes was maintained at 1 mm. A 30 W solar panel was used for power generation and a 40 Ah lithium polymer battery was used to provide electricity during periods of insufficient sunlight. The voltage applied to the reactor was regulated through the adjustment of input voltage from 3 to 6 V, which was then amplified to a range of 17–27 kV by a Tesla coil (high voltage amplifier; ModuleMe Electronic, China). A working frequency of 0.3 Hz, along with a 50% duty cycle, was used in the power module to ensure stable output and minimize internal heat generation.

2.2 Seeds and PAW preparation

The spinach (*Spinacia oleracea* line 08-280, 08-415, F415) seeds used in this study were provided by the Vegetable Research Station of University of Arkansas (3810 Thornhill Street, Alma, AR, USA), which had been sealed in zipper bags and stored at 4 °C in a cool room for 23 years. Prior to the main experiment, the seeds were surface-sterilized by soaking in the ethanol solution (~90%) for 1 min followed rinsing with deionized water. These seeds were then immersed in the PAW under

experimental conditions for 12 h to test for potential induction of germination. Only seeds that were visibly intact were selected, ensuring that any broken, crushed or infected seeds were excluded from the experiments. Polystyrene petri dishes (100 mm) and 15 mm in height were used for germination test. The base of each dish was covered with Whatman filter paper (No. 28310-048), 2 mL of untreated or plasma-treated tap water and 30 spinach seeds were added. The dishes were covered to maintain a controlled environment, with lids opened for approximately 30 min each day to ensure adequate air exchange and prevent over-saturation. The experiments were conducted under constant conditions at 23.8 °C with a photoperiod of 12 h light/12 h dark. A further 2 mL of untreated or plasma-treated water was added daily to compensate for evaporative losses.

For PAW preparation, the tap water was exposed to plasma for various durations under atmosphere conditions, viz., 17 kV for 5, 10, and 15 min (PAW17-5, PAW17-10, and PAW17-15), and 22 and 27 kV for 10 min (PAW22-10 and PAW27-10). The PAW was used immediately after activation. Each of the five plasma treatments and one untreated control were replicated three times, totaling 540 seeds across 18 experimental units.

2.3 Analytical methods

The seed germination rate, and shoot and root length were recorded daily at 10:00 AM for 6 days. Nitrite and nitrate were measured using Hach vials (TNT 822 and TNT 872, Hach Company, Loveland, CO, USA) with a Hach DR 3900 spectrophotometer according to the manufacturer's instructions. The ORP was measured using an EZO-ORP kit from Atlas Scientific (Long Island City, NY, USA), and the pH

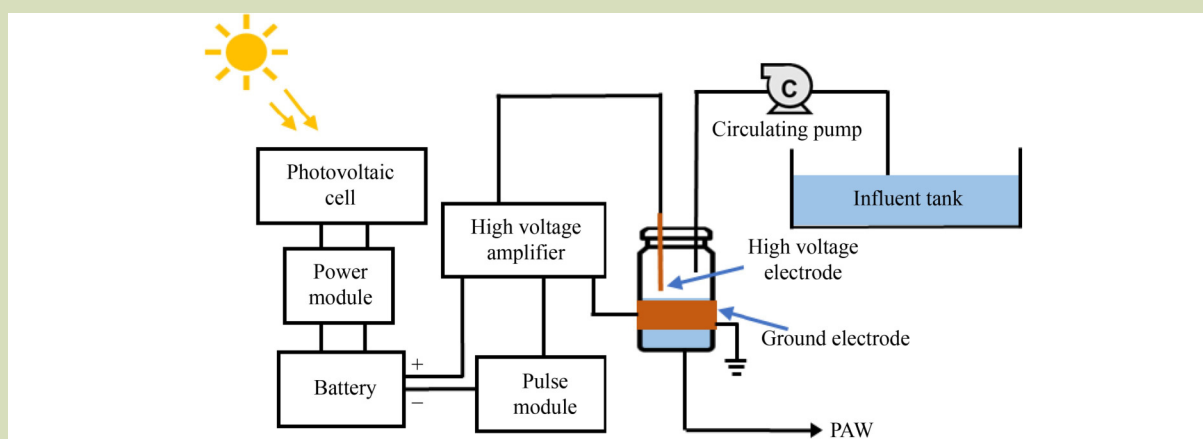


Fig. 1 Schematic of the solar-powered corona dielectric barrier discharge (cDBD) reactor used in this study.

of the solution was determined using a PHS-25 digital-display pH meter (XL 600, Fisher Scientific, Hampton, NH, USA). The pH measurements were taken immediately after the activation of PAW and could be affected by the elevated temperature, leading to the enhanced ion mobility and the increased molecular dissociation^[17], a mandatory recalibration process was performed using the Nernst's equation:

$$\text{pH} = \frac{E_0 - E}{2.3 \frac{R \times T}{F}} \quad (1)$$

where, F is the Faraday constant, E is the measured electrode potential, T is the temperature, and R is the universal gas constant. The standard potential (E_0) and the coefficient were estimated using a two-point calibration in buffer solutions at pH 4.01 and 7.00.

t -tests were used to compare the germination rates between PAW and tap water treatments and the p -value was used to assess statistical significance.

3 Results and discussion

3.1 Physiochemical properties of PAW

Tables 1 and 2 shows the pH, ORP, nitrite and nitrate content of the tap water (used as the control) and PAW treated solution at different times at 17 kV and different voltages for 10 min. Overall, the pH decreased with longer treatment times and higher plasma discharge voltages, whereas the ORP, nitrite and

nitrate concentrations increased correspondingly. Additionally, the impact of voltage on these parameters was more substantial than that of treatment time, indicating a greater effect of voltage on pH, ORP, nitrite and nitrate concentrations.

The pH of the environment in which a seed germinates impacts germination success. Most seeds germinate more effectively at a slightly acidic to neutral pH for optimal enzyme activity. Enzymes essential for breaking down food reserves in the seed are pH-sensitive, requiring an optimal pH for efficient operation. Highly acidic environments hinder the synthesis and activity of these enzymes, compromise seed coat integrity through dissolution or pathogen-induced perforation, and impede essential biochemical pathways^[18]. According to Jensen and Thomas^[19], the majority of plants grow best in a pH range of 6.5–7.5, which is considered optimal for root development. However, low pH can significantly impede seed germination by damaging the seed coat, adversely affecting the activity of enzymes essential for seed growth, increasing concentrations of toxic metal ions, disrupting the balance of soil microbial communities and causing hormonal imbalances in seeds. Therefore, the pH reduction caused by NTP dischargers can be detrimental. To address this, modifying the NTP discharger to minimize acid production is important, involving optimization of operating voltage and frequency, using inert gases as feedstock, and controlling of treatment duration and environmental conditions. Compared to other studies, where pH dropped to 3 within minutes, the present study recorded a relatively similar hydroxide ion concentration, calculated using the ionic product, but only minor decreases in pH were observed (from 7.37 in the control

Table 1 Quantification of pH, oxidation-reduction potential (ORP), NO_2^- , and NO_3^- in PAW treated with different time at 17 kV

Treatment time (min)	pH	ORP (mV)	NO_2^- (ppm)	NO_3^- (ppm)
0	7.37	262	0.048	0.797
5	7.22	269	2.87	3.64
10	7.20	275	4.05	5.19
15	7.18	284	6.45	6.45

Table 2 Quantification of pH, oxidation-reduction potential (ORP), NO_2^- , and NO_3^- in PAW treated with different voltage (10 min)

Treatment voltage (kV)	pH	ORP (mV)	NO_2^- (ppm)	NO_3^- (ppm)
Untreated	7.37	262	0.048	0.797
17	7.20	275	4.03	5.19
22	6.9	291	7.15	11.1
27	6.86	292	8.65	13.5

to 7.18 and 6.86 in the PAW17-15 and PAW27-10, respectively). This result is similar to the findings of Judée et al.^[20] on the stability pH after plasma treatment, with some minor variations that could be due to differences in the setup of the plasma device.

The oxidizing and reducing capabilities of the solutions were assessed using their ORP, which increased with the generation of reactive oxygen and nitrogen species by the NTP discharger. While these reactive species can be beneficial at certain concentrations, excessively high ORP can induce oxidative stress, potentially damaging the cellular structures of seeds and consequently inhibiting or delaying germination^[21]. In addition, the World Health Organization has established a standard for drinking water, where an ORP of 650 mV is considered sufficient for immediate bacterial disinfection^[22]. This standard implies that while a higher ORP is effective for disinfection, moderate ORP is generally preferable for processes like seed germination to avoid the negative effects of excessive oxidative stress.

Both nitrite and nitrate are important for enhancing seed germination. As outlined by Hendricks and Taylorson^[23], the measured enzymatic activities and the observed sensitivity of hemoproteins to inhibition by various compounds suggest that nitrites enhance seed germination through the inhibition of hydrogen peroxide breakdown by catalase (CAT). Concurrently, nitrate at low concentrations acts as both a nutrient and a signaling molecule, undergoing assimilation first into nitrite and then into ammonium, an important step for amino acid synthesis^[24]. This dual function highlights the significance of nitrite and nitrate, and both should be adjusted through controlling operational parameters of NTP dischargers. It has been found that higher voltage settings are more conducive to the formation of nitrates than nitrites, so this is an important factor in PAW preparation. For example, the nitrate content in PAW generated at 27 kV for 10 min ($13.5 \text{ mg}\cdot\text{L}^{-1}$) doubled that produced at 17 kV for 15 min ($6.45 \text{ mg}\cdot\text{L}^{-1}$). This indicates that the operational parameters of NTP dischargers are important and that a higher voltage tends to favor the accumulation of desirable nitrate concentrations in PAW. Also, higher nitrate concentrations compared to nitrites in PAW are most likely due to ozone-induced nitrite-to-nitrate conversion^[20]. This observation aligns with a study by Liu et al.^[25], who attributed 90% of the nitrite reduction in PAW to excess ozone.

3.2 Seed germination

The germination rate of spinach is significantly affected by

both the voltage and the treatment duration of the NTP discharger, with all PAW treatments surpassing the performance of the untreated control. Specifically, longer exposure times generally lead to increased germination. Also, while there was no germination on day 3 in lines 08-415 and F415 (Fig. 2(c, e)), germination occurred with PAW treatment at 17 kV for 15 min in 08-415, and for 5, 10, and 15 min in F415. However, there was increased germination rates of lines 08-280 and 08-415 with longer PAW treatment at 17 kV (Fig. 2(a, c)), the highest rates were achieved at 15 min reaching 60% and 40%, respectively. However, line F415 deviated from this trend. The highest germination rate for line F415 was 30% at a shorter PAW exposure of 10 min at 17 kV (Fig. 2(e)). This variation indicates that different seed lines have specific optimal germination conditions, most likely due to their unique nutritional requirements or differential responses to PAW treatment. By day 6, the germination with PAW peaked at 60% for 08-280 (PAW17-15), 40% for 08-415 (PAW17-15) and 30% for F415 (PAW17-10). These represent substantial improvements over the control, being an increase of about 62% for 08-280, 135% for 08-415 and 130% for F415 over the control germination rates of 37%, 17%, and 13%, respectively. The effectiveness of PAW in enhancing germination is further substantiated by the notably low *p*-values derived from the *t*-test, all of which are well below the 0.01 threshold, indicating a statistically significant improvement in germination rates attributable to PAW treatment.

The germination of spinach seeds was also significantly affected by the voltage applied during PAW treatment, with PAW22-10 giving 60% germination with line 08-280 (Fig. 2(b)) and 33% with line 08-415 (Fig. 2(d)), and PAW17-10 giving 30% with line F415 (Fig. 2(f)). These differences can be attributed to the optimal generation of reactive oxygen and nitrogen species (ROS and RNS) at specific NTP discharger voltages. These reactive species are important for stimulating germination without the negative effects that come with higher voltages, such as more ROS and lower pH, which can lead to seed surface erosion or an excessive buildup of reactive species. This highlights the importance of finding a balanced approach to PAW treatment that considers both voltage and duration to maximize benefits across various seed lines.

Notably, the impact of voltage on germination aligns with the previously discussed effects of treatment duration. As treatment time increases or voltage rises, parameters such as pH, ORP, and concentrations of NO_2^- and NO_3^- tend to decrease, affecting seed germination. Accordingly, the optimal germination rates observed with extended PAW treatment at 60% for 08-280 (PAW17-15), 40% for 08-415 (PAW17-15), and 30% for F415 (PAW17-10) were consistent with the

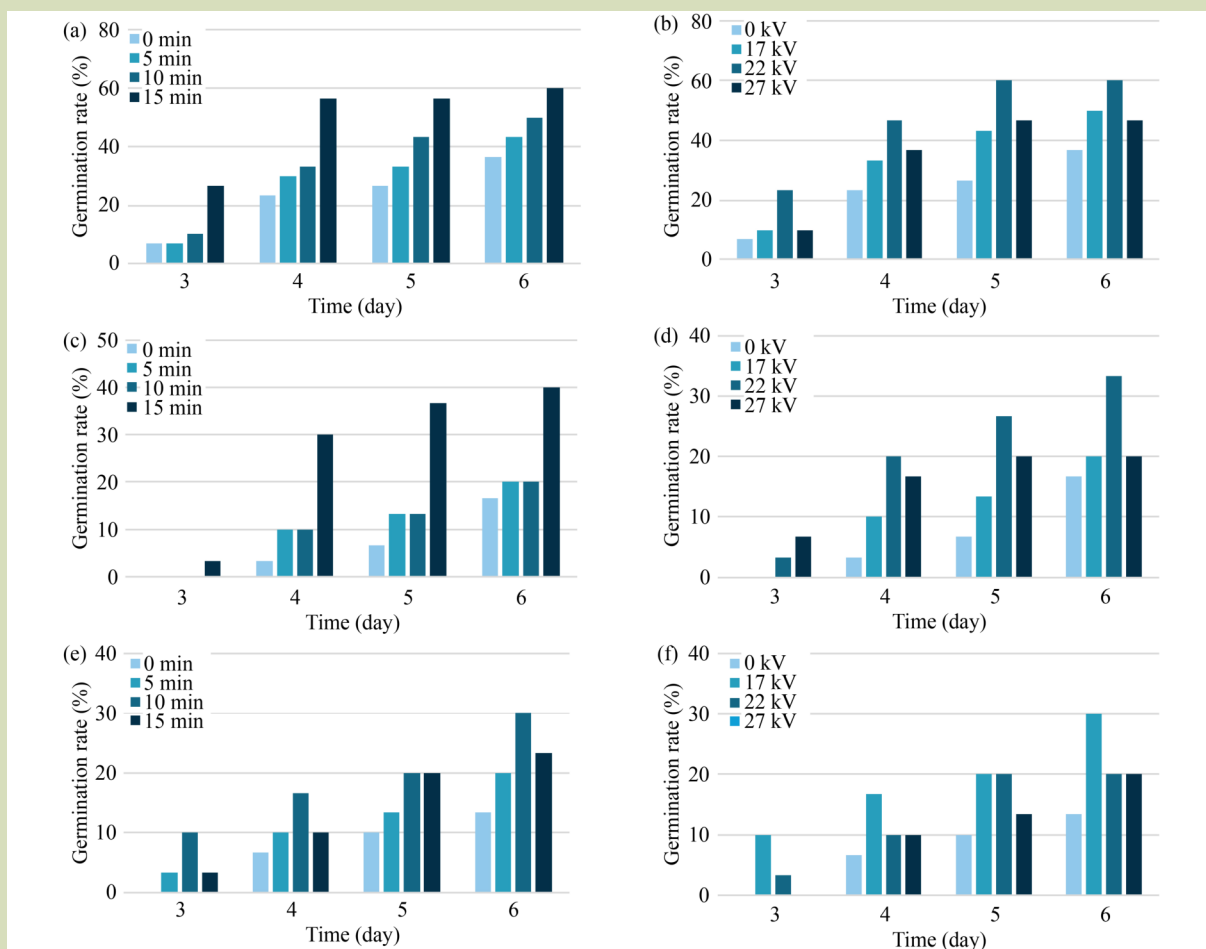


Fig. 2 Germination results for spinach seeds for lines 08-280 (a, b), 08-415 (c, d), and F415 (e, f) on days 3–6. (a, c, e) Time effects at 17 kV for 0, 5, 10, 15 min; (b, d, f) voltage effects with 0, 17, 22, 27 kV for 10 min.

patterns seen with voltage adjustments, where PAW22-10 treatment yields similarly high germination rates for lines 08-280 and 08-415, and PAW17-10 does so for F415. This consistency between the effects of treatment duration and voltage emphasizes the complex interplay between these factors and their collective influence on the efficacy of PAW treatment in promoting seed germination.

A representative petri dish from control and PAW17-5 at the end of the germination phase are shown in Fig. 3. It is obvious that the seedlings from the PAW17-5 treatment had a higher germination rate and greater vigor (max length of 5.8 mm) compared to the control (max length of 26 mm). The p -values indicated a significant difference in germination rates between PAW treated and untreated seeds. This is particularly noteworthy given the age of the seed (23 years), for which the germination rate was previously less than 20%. The PAW

treatment has evidently resulted in more robust and healthy seedlings. It is possible that nitrates generated during plasma activation served as an alternative source of nitrogen that enhances plant growth. Similar phenomena have been reported by Takaki et al.^[26], who reported that PAW significantly boosted the growth of *Brassica rapa* var. *perviridis* over 28 days, with plants in 20-min treated PAW reaching 90 mm, over twice the length of the 40 mm in the control, indicating that the reactive nitrogen species in PAW functioned as an effective fertilizer. Similarly, Judée et al.^[20] found that PAW irrigation increased lentil seedling lengths by 34% and 128% after 3 and 6 days, respectively, compared to untreated water, with other studies reporting comparable effects. The consistent observations across various studies highlight the potential of PAW as a sustainable enhancer for plant growth, where it compensates for diminished natural germination rates by providing a nutrient boost through reactive nitrogen species.

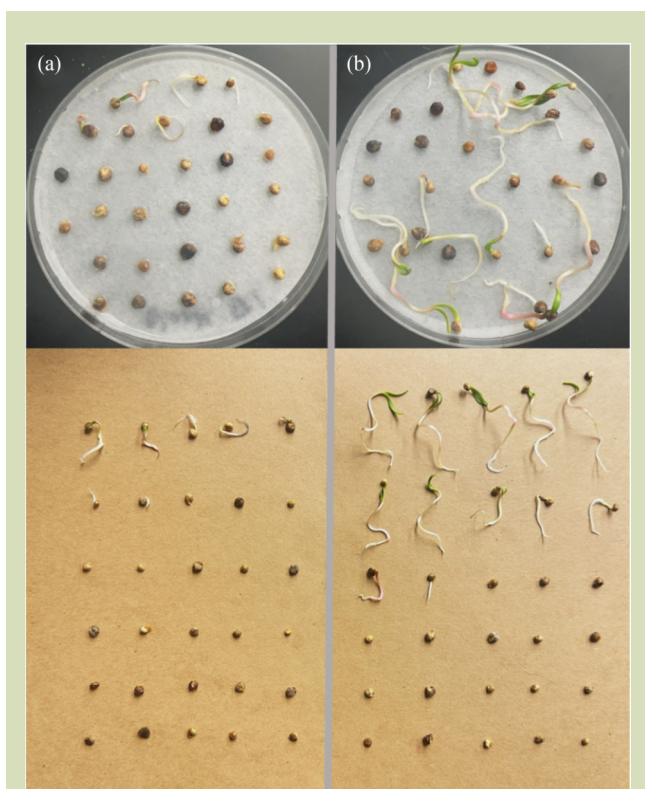
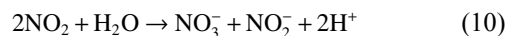
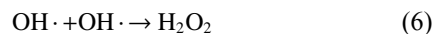
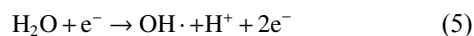
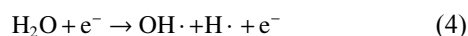
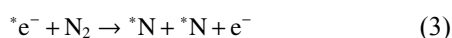
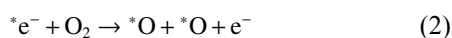


Fig. 3 Spinach (08-280) germination and seedling growth after 7 days for control (a) and PAW-17-5 (b).

3.3 Mechanisms

Despite the challenges associated with analyzing the chemical processes of plasma interactions with tap water, which vary in salt concentrations and organic impurities, the primary mechanism of the solar-powered plasma device used for seed germination remains clear and well-defined. This mechanism involves the generation of reactive species through NTP. This process starts with electron collisions with neutral molecules, leading to the formation of primary reactive species such as ionized and excited molecules, atomic nitrogen and oxygen (Eqs. (2)–(3)). These species rapidly evolve into secondary reactive species including H_2O_2 , nitric oxide, and ozone (Eqs. (4)–(9)), which would dissolve into the liquid phase to become a more stable form (Eqs. (10)–(12)). These stable species, which include O_3 , H_2O_2 , NO_2^- , NO_3^- , and peroxyxynitrite, and have a longer lifespan (ranging from milliseconds to several days), and are important for enhancing seed germination as discussed below.



PAW can improve seed germination rates by increasing the surface wettability of the seeds^[27], killing bacteria, and pathogens present on the seed surface^[28], softening the seed coat and stimulating the growth of hypocotyl and radicle^[29]. This enhanced germination and growth from PAW treatment can be further understood by examining its impact on the fundamental stages of seed development. The germination process involves two critical phases: the primary cell elongation in the axial part of the embryo, and cell division, either simultaneous or delayed, in the radicle meristem^[30]. Research indicates that both critical phases can be affected by PAW through the modulation of gibberellins (GA), abscisic acid (ABA), and CAT. GA influences the primary cell elongation phase, and is essential for breaking seed dormancy and initiating growth by stimulating cell elongation within the embryo^[31]. In contrast, ABA generally acts to maintain seed dormancy and inhibit germination, regulating stress responses to prevent premature germination under unfavorable conditions^[32]. Meanwhile, CAT activity becomes particularly relevant during the cell division phase in the radicle meristem, where it helps manage oxidative stress, important for protecting actively dividing cells and supporting healthy seedling development^[33].

Based on this understanding, the role of ROS and RNS in several signaling pathways involved in the seed germination was investigated. First, ROS and RNS could modulate ABA and GA transduction pathways and help in controlling numerous transcription factors and altering the properties of specific proteins through carbonylation^[34]. For example, Grainge et al.^[35] reported that PAW could facilitate the release of physiological seed dormancy in *Arabidopsis thaliana* through a synergistic interaction between plasma-generated reactive species (NO_3^- , H_2O_2 , $\cdot\text{NO}$, and $\cdot\text{OH}$) and signaling pathways that target GA and ABA metabolism. This interaction also alters the expression of genes responsible for cell wall

remodeling, vital for germination. To be more specific, they found that when the seeds were treated with air-PAW, there was an early upregulation of genes like GA3OX1 (involved in bioactive GA biosynthesis) and CYP707A2 (ABA degradation), along with the downregulation of NCED2 and NCED9 (ABA biosynthesis). In addition, PAW treatment can also enhance the CAT activity in the roots of the grown plants^[36]. This increased CAT activity contributed to the upregulation of various physiological processes, resulting in improved germination, growth, and overall development of the target seeds. Similar research has been conducted by Puač et al.^[37], who found that the presence of H₂O₂ in PAW was important in activating the CAT genes in seeds. This activation led to the synthesis of new proteins, which was observed to significantly enhance the germination of *Paulownia tomentosa* seeds. Those reactive species in PAW can actually be generated and released by mitochondria within the cell, which are regarded as the primary sites of production^[38]. It has been found that RNS are more effective in stimulating the germination rate at higher concentrations, while an increase in ROS concentrations is associated with longer shoot growth. Thus, the presence of additional reactive species present in PAW could significantly boost the seed germination rate^[39].

4 Conclusions and perspectives

This study addressed the important issue of declining germination rates in aged seeds, a common problem in agriculture. The solar-powered corona dielectric barrier discharge (cDBD) microreactor used was capable of generating PAW efficiently and in an environmentally sustainable manner. Physicochemical analysis revealed that longer treatment times and higher voltages decreased pH and

increased ORP, nitrite and nitrate concentrations in the PAW. The optimal PAW treatment (PAW17-15), applied to 23-year-old spinach seeds (line 08-415), led to a remarkable 135% increase in germination rates compared to the control. This study also detailed the changes in physicochemical properties of PAW and their effects on seeds at a molecular level, demonstrating how PAW treatment not only improved germination by presumably modulating GA and ABA but possibly also enhanced early seedling growth through increased CAT activity.

These findings highlight the effectiveness of this solar-powered cDBD microreactor in enhancing the viability of aged seeds, offering a sustainable and cost-effective solution to support global agricultural food production. This technology could also serve as an on-site tool for farmers, promoting the adoption of more resilient agricultural practices. However, while PAW has proven to enhance seed germination by modifying seed surface properties and facilitating the formation of reactive species that break seed dormancy, the complex interactions among PAE, seed germination, and plant growth need to be fully understood. The variability in plant species, growth phases and environmental conditions can significantly influence the outcomes of plasma treatments. Also, the potential scaling of such plasma devices for large-scale agricultural operations remains to be tested including work on different seed types across various conditions as these will impact the consistency and reliability of the results. Additionally, the long-term impacts of plasma-treated seeds on crop yield and quality need further evaluation to ensure farmer adoption. Further research and development are essential to address these limitations, enhancing the feasibility of plasma technology in agriculture and providing a more comprehensive understanding of its benefits and constraints.

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Compliance with ethics guidelines

Yiting Xiao, Yang Tian, Haizheng Xiong, Ainong Shi, and Jun Zhu declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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