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Effects of Plasma-Activated Water on Wheat: Germination and Seedling Development

Wafaa Abdulrazzaq Abdullah
Ministry of Education, Al-Karkh Second Directorate, wafaaphy@yahoo.com

Hadeel O. Ismael
Department of Physics - College of Science - University of Baghdad, Baghdad - Iraq

Duaa A. Uamran
Department of Physics - College of Science - University of Kerbala, Karbala - Iraq, duaa.fo1984@uokerbala.edu.iq

Hammad R. Humud
Department of Physics - College of Science - University of Baghdad, Baghdad - Iraq

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Abstract

The food sector must contend with issues such as pathogen resistance to some of the available chemical agents, environmental pollution, and climate change to provide healthy food for livestock and people. The application of atmospheric pressure plasma jets (APPJs) is one potential solution for such problems. Plasma is appropriate for effective surface decontamination regarding food products and seeds, surface decontamination, and achieving improved agricultural production yields. The impact of plasma-activated water (PAW) produced by plasma jet discharge (PJD) system on in vitro-cultivated wheat seeds is examined in this work. For this aim, a plasma jet system was constructed with a total power of 60W, a frequency of 20kHz, and an AC voltage sine wave ranging between 0 and 12 kVA from peak to peak. Through directly activating 60 cc of distilled water (DW) with 20 cc of plasma containing varying quantities of reactive oxygen as well as nitrogen species (RONS) and pH, such method uses 2.5 liters of argon gas every minute. To produce PAW, DW has been exposed to PJD for 20 minutes. Three hundred wheat seeds have been tagged and split equally into five groups for this study. Active water was applied to three groups of seeds. The last group received simply DW treatment, the fourth group's seeds were placed in DW before being exposed directly to plasma. In PAW, the seeds were soaked for a whole day. Following a 24-hour period, fifteen germination plates—three for each group—were used to plant the seeds. Following soaking, the seeds were observed for three days, and the dishes were placed in a dry room at room temperature and administered DW as directed. Monitoring was done using germination indicators and parameters, such as the germination rate (GR) and the percentage of germination (Gp). Following its plasma treatment, DW resulted in notable modifications to its chemical parameters and physical characteristics. In the case when seeds were soaked in plasma-treated water, the rate of germination (metrics of growth, like shoot/seedling length as well as seeds' imbibition rate) rose. The seeds that were solely soaked in DW produced a higher germination yield. Since RONS in PAW interacted directly with the surface of the seed, increasing the germination rate and water and nutrient uptake, group S4 produced the greatest results when the seeds have been directly exposed to plasma..

Keywords

Germination, plasma-activated water, plasma jet, wheat seeds, reactive oxygen and nitrogen species.

RESEARCH PAPER

Effects of Plasma-activated Water on Wheat: Germination and Seedling Development

Wafaa A. Abdullah [a], Hadeel O. Ismael [b], Duaa A. Uamran [c,*], Hammad R. Humud [b]

[a] Ministry of Education, Al-Karkh Second Directorate, Iraq
[b] Department of Physics, College of Science, University of Baghdad, Baghdad, Iraq
[c] Department of Physics, College of Science, University of Kerbala, Karbala, Iraq

Abstract

The food sector must contend with issues such as pathogen resistance to some of the available chemical agents, environmental pollution, and climate change to provide healthy food for livestock and people. The application of atmospheric pressure plasma jets (APPJs) is one potential solution for such problems. Plasma is appropriate for effective surface decontamination regarding food products and seeds, surface decontamination, and achieving improved agricultural production yields. The impact of plasma-activated water (PAW) produced by the plasma jet discharge (PJD) system on in vitro-cultivated wheat seeds is examined in this work. For this aim, a plasma jet system was constructed with a total power of 60 W, a frequency of 20 kHz, and an AC voltage sine wave ranging between 0 and 12 kVA from peak to peak. Through directly activating 60 cc of distilled water (DW) with 20 cc of plasma containing varying quantities of reactive oxygen as well as nitrogen species (RONS) and pH, such a method uses 2.5 L of argon gas every minute. To produce PAW, DW has been exposed to PJD for 20 min. For this study, three hundred wheat seeds have been tagged and split equally into five groups. Active water was applied to three groups of seeds. The last group received simply DW treatment, the fourth group's seeds were placed in DW before being exposed directly to plasma. In PAW, the seeds were soaked for a whole day. Following 24 h, fifteen germination plates—three for each group—were used to plant the seeds. Following soaking, the seeds were observed for three days, and the dishes were placed in a dry room at room temperature, and DW was administered as directed. Monitoring was done using germination indicators and parameters, such as the germination rate (GR) and the percentage of germination (Gp). Following its plasma treatment, DW resulted in notable modifications to its chemical parameters and physical characteristics. In the case when seeds were soaked in plasma-treated water, the rate of germination (metrics of growth, like shoot/seedling length as well as seeds' imbibition rate) rose. The seeds that were solely soaked in DW produced a higher germination yield. Since RONS in PAW interacted directly with the surface of the seed, increasing the germination rate and water and nutrient uptake, group S4 produced the greatest results when the seeds were directly exposed to plasma.

Keywords: Germination, Plasma-activated water, Plasma jet, Wheat seeds, Reactive oxygen and nitrogen species

1. Introduction

In good production and agriculture, cold atmospheric pressure plasma (CAPP) is utilized somewhat extensively for treating seeds and plants as well as a more environmentally friendly method of water desalination, remediation, and decontamination [1—3]. Current research on CAPP's impacts focuses on plasma application to the plants as one of the potential ecological solutions for food production increase, in addition to water purification. The challenges the food industry has in providing healthy food for livestock and people include environmental pollution, climate change, and growing pathogen resistance to the available chemical agents [4,5]. The application of CAPP is one promising solution for such issues. Plasma can be administered to a wide range of agricultural products both before and after harvest thanks to the variety of accessible plasma sources as well as the

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* Corresponding author.
E-mail addresses: duaa.fo1984@uokerbala.edu.iq, duaa_fo1984@yahoo.com (D.A. Uamran).

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treatment's ease of use. The primary purpose of preharvest application is to enhance germination through seed treatment [6,7]. Preharvest plasma treatment has several benefits, such as improved germination [6,8], surface decontamination [6,9], quicker growth [10], and improvement of related favorable biochemical and physiological characteristics [11]. Various seeds treated with the plasma generated by the exact source of the plasma, as in this paper, have also shown such effects [12,13]. The production of different gaseous and aqueous reactive oxygen and nitrogen species (RONS) is a feature of plasma discharge. Many processes in plants and seeds, including growth, germination, stress response, and development, are influenced and controlled by RONS. The primary species thought to be in charge of promoting the seed germination and growth of the plants that have been irrigated with plasma-activated water (PAW) are RONS, more especially nitrites (NO_2^-), hydrogen peroxide (H_2O_2), and nitrates (NO_3^-).

Plasma-activated water (PAW) contains reactive oxygen and nitrogen species (RONS), crucial in enhancing wheat germination and seedling development. These species include hydrogen peroxide (H_2O_2), nitrate (NO_3-), nitrite (NO_2-), superoxide radicals $\cdot O_2-$), and hydroxyl radicals $\cdot$OH). The interaction of RONS with biological systems can be complex, influencing both plant growth promotion and stress responses. Hydrogen Peroxide can increase respiration, facilitate water entry into seeds, and oxidize germination inhibitors, thereby enhancing seed germination [14].

Nitrate is a plant nutrient source but also influences hormone signaling pathways like abscisic acid/gibberellic acid balance to reduce dormancy. RONS can modulate plant hormone levels by interfering with signaling pathways such as abscisic and gibberellic acid. This interference accelerates seed sprouting by reducing latency periods. PAW enhances antioxidant enzyme activity in some cases but may suppress it in others, depending on the concentration of RON present [15]. This modulation helps protect plants from oxidative stress. Increased availability of nutrients like nitrates from PAW supports better growth parameters, such as increased photosynthetic pigments and soluble protein content in wheat seedlings.

Pathogens on the surface of seeds could be eliminated by plasma through low amounts of oxidative stress [13]. As signal molecules, RONS could alter seed germination signal pathways, and plasma could affect their intracellular concentration. The working gas, plasma source, type of treated seed, and surface morphology could all affect how effectively plasma is treated [16]. Because of such factors, establishing general plasma treatment conditions for various seeds is impossible. As was noted in numerous investigations [17,18], the optimal plasma treatment time varies by seed species and is likely influenced by parameters like seed type, surface hardness, size, and embryo location. But this isn't the only issue with plasma treatment. The exposure time required for pathogen inactivation might occasionally be excessively long, harming the seeds and lowering germination. It was demonstrated that prolonged plasma exposure to seeds seriously damages their morphology, which lowers germination. About 700 million tons of wheat are harvested each year for animal feed and human consumption, making it one of the most extensively cultivated crops in the world [19]. Global wheat consumption has steadily risen due to expanding family wealth and population growth in low- and middle-income countries (LMICs) [20,21]. Therefore, improved wheat germination and crop production advancements are required. With uses in agriculture and medicine, PAW exhibits exceptional biotic activity [15].

A plasma jet system designed for this purpose with a 0–12 kVA peak-to-peak AC voltage sine wave, a total power of 60 W, and a 20 kHz frequency was used for identifying treatment conditions for wheat seeds. Through directly activating 60 ccs of DW with 20 ccs of plasma with varying RONS concentrations and pH, this system employs 2.5 L of argon gas per minute for treating the water while preserving its beneficial properties (such as increased germination). Determining the concentration of RONS in the PAW for enhanced wheat germination is crucial in finding suitable uses for this technology in agriculture, as RONS is one of the primary active plasma components.

2. Seeds

The impact of PAW on seed germination and seedling growth has been examined in vivo and in vitro. The wheat seeds were sourced from farms in the Arab Al-Jabour area, a rural Iraqi town situated north of Mahmoudiyah district and south of Baghdad.

3. Germination

The ratio of the number of the seeds that germinated to total number of the seeds in each Petri dish was utilized in order to calculate the percentage of germination. In the case when at least 1 mm of the coleoptile the sheath that protects a young shoot tip in a grass or cereal and radicle the portion of a plant embryo that grows into the primary root emerged from the seed, it was deemed to have germinated.

4. Experimental methods

Fig. 1 illustrates the water treatment system. The process of energizing water was developed using a plasma system, which was designed for this task and consisted of a power supply that generates a sine wave of 20 kHz frequency and can generate a high capacity AC voltage of up to 12 kV. An electrode composed of coiled aluminum was attached to a high voltage in a Teflon tube with an internal diameter of 5 mm and was positioned 1 cm from the tube's end. Additionally, it was connected to the argon gas regulator, which offers a 2.5 L/min flow rate. This gas flow rate indicates that the flow is laminar.

Fig. 2a depicts the plasma system in operation. The plasma activated the water in the 30-mL container. The hard plastic cylindrical vial is 3.5 cm in diameter and 3 cm in depth. There was approximately 1.5 cm of headspace when the plasma was generated in the Teflon tube. After 20 min of exposure to plasma at a 2.5 L/min flow rate, the DW has been activated. The plasma system operating with wheat seeds inside the PAW is seen in Fig. 2b. The end of the Teflon tube in which the plasma was produced was 2 cm from the water's surface in order to get various concentrations of NO_3, NO_2, and H_2O_2 (i.e., RONS).

NO_3, NO_2, H_2O_2, and pH Concentrations were measured using test strips (Bartvation, USA). The test was accomplished by immersing the strip in activated water for several seconds and removing excess water. After 30 s, the strip color is compared with the standard color, as in Figure (3). A pH meter was used for measuring the pH. A remote infrared thermometer was used to take the temperature. The pH of PAW often decreases with extended exposure time due to the accumulation of acidic species like nitrites (NO_2^-) and nitrates (NO_3^-). However, some studies show a non-linear change where the pH may initially increase before stabilizing.

In this work, 300 wheat seeds were chosen and divided into 5 groups, each containing 60 seeds. The chosen seeds were of one size, healthy, and capable of germination. After washing them well, these seeds were sterilized by soaking them in chlorine diluted with distilled water for 10 min. After that, the seeds were washed and dried.

These seeds were randomly divided into five groups (S1, S2, S3, S4, and control), which represent the DW without exposure to plasma, and treatments at the different concentrations of NO_3, NO_2, and H_2O_2 (RONS) shown in Table 1. The RONS concentrations were obtained by diluting the activated water. In the first group (S1), 20 ml of activated water was used, while in the second group (S2), 20 ml of PAW was added to 20 ml of DW. As for the

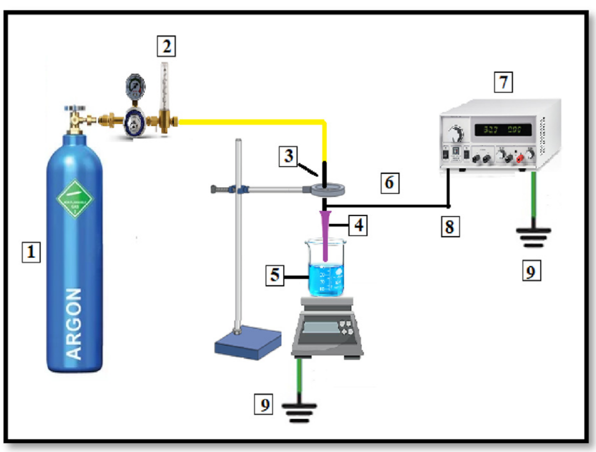

Fig. 1. Plasma system developed for PAW used for seed germination consisting of (1) Argon gas, (2) flow meter, (3) Teflon tubing, (4) plasma jet, (5) PAW, (6) aluminum tape electrode, (7) AC power supply, (8) high-voltage electrode, and (9) ground electrode.

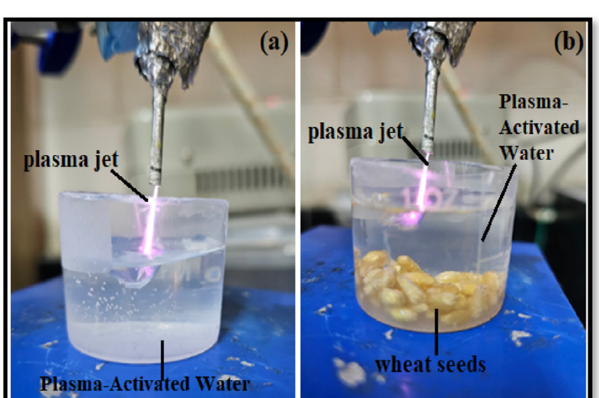

Fig. 2. (a) Plasma system activating water at a (2.5 L/min) gas flow rate, (b) plasma system activating water containing wheat seeds.

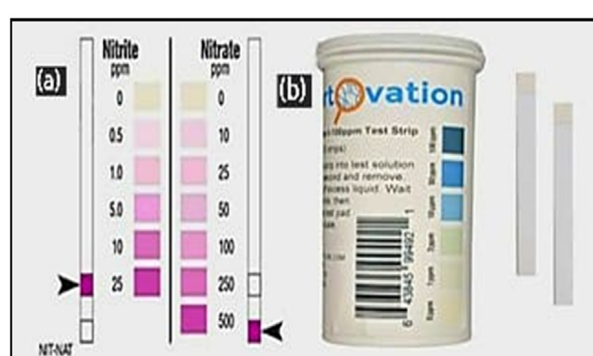

Fig. 3. Strips used to measure the NO_2, NO_3, and H_2O_2 concentrations in ppm.

Table 1. Concentration levels of NO_3, NO_2, and H_2O_2 (ppm) and pH in the plasma-activated water. (S1, S2, S3, S4, and control), which represents the DW without exposure to plasma and treatments at the different concentrations of NO_3, NO_2, and H_2O_2 (RONS).

Group	NO_2 (ppm)	NO_3 (ppm)	H_2O_2 (ppm)	Total Reactive species (RONS) (NO_2+NO_3+H_2O_2)	pH
S1	7	100	100	207	2.8
S2	4	50	50	104	5
S3	5	66	66	137	4
S4	25	250	100	375	3
Control	0	0	0	0	7

third group (S3), 20 ml of activated water was mixed with 10 ml of DW. The total RONS concentrations of S1, S2, and S3 were 207, 104, and 137 ppm, respectively. The control group consisted of DW not activated with plasma. Finally, for group S4 (Fig. 2b), the seeds were placed in DW, exposed to plasma, and germinated. The total RONS concentration was 375 ppm.

The seeds in groups S1, S2, and S3 (Fig. 2a) were soaked with PAW for 24 h and then dried and placed in specially prepared germination plates. The seeds of each group were distributed uniformly on three plates, each containing 20 seeds. The seeds were set on a piece of porous cloth to keep them moist, and the dishes were covered with a nylon cover for two days before the cover was taken off on the third day. The seeds were sprayed six times a day throughout the first three days of germination. The plates were set up with artificial lighting day and night, a steady temperature, and a moderate to average humidity level. All of the biological changes to the seeds were noted and documented, as was the number of seeds that germinated on the first, second, and third days of germination. The S4 underwent the same procedures as the S4, except this group's seeds were exposed to plasma directly for 20 min before being dried and put in the germination plates.

Longer exposure times generally lead to higher concentrations of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), hydroxyl radicals (·OH), and reactive nitrogen species (RNS) like nitrate ions. This increase enhances the oxidation-reduction potential (ORP) of PAW. Different gases used in plasma generation can produce varying types and amounts of RONS. For example, air or nitrogen-rich mixtures generate more nitrogen-based RNS than pure oxygen or helium plasmas. The choice of gas can affect the final pH by influencing the production rates of acidic species like NO_2- and NO_3-.Gas composition affects not only the initial concentration but also the stability over time for particular reactive species, which is crucial for maintaining efficacy during storage or application. Understanding these variations is essential for tailoring PAW properties for specific applications by adjusting both plasma treatment duration and gas composition accordingly [22,23].

In this study, we employed distilled water as our negative control baseline against which we compared the effects of PAW on wheat germination and seedling development. We justified our choice of statistical analyses by first assessing data distribution.

5. Result and discussion

Table 1 exhibits the concentrations of NO_3, NO_2, and H_2O_2 and the pH of the PAW used for soaking the wheat seeds.

Throughout the germination period, which is when the seedling begins to sprout from seed surface, the wheat seeds were continuously soaked in an equal quantity of PAW for three days in a row. As seen in Table 2, soaking the seeds with PAW aided in accelerating germination throughout the first three days of germination.

The number of germinating seeds depended on the RONS. Table 3 shows the average length of roots and vegetative part length of seeds on the third day of germination for the five groups.

Table 2. Number of germinating and non-germinating seeds during the three days of germination.

Groups	Total number of seeds	Number of germinated seeds wheat on the first day	Number of germinated seeds wheat on the second day	Number of germinated seeds wheat on the third day	The number of non-germinating seeds wheat at the end of germination
S1	60	57	58	59	1
S2	60	60	60	60	0
S3	60	55	57	59	1
S4	60	56	60	60	0
Control	60	54	55	55	5

Table 3. Average length of roots and vegetative of seeds on the third day of germination.

Groups	Root length (cm)	Vegetative part length (cm)
S1	7	8.2
S2	8.6	9.3
S3	8	9.1
S4	7.5	9.5
Control	3.5	4.4

Understanding the chemical alterations brought on by plasma discharge in water is essential to comprehending how PAW affects germination as well as plant growth. The discharge mostly creates NO_2 and NO when it is created in ambient air. When hydroxyl radicals ($\cdot OH$) come into contact with water, they quickly recombine to form hydrogen peroxide, or H_2O_2 (Eq. (1)). The water becomes acidic as a result of gaseous NO and NO_2 dissolving in it and producing nitrates (NO_3^-) and nitrites (NO_2^-). Plasma-activated water may alter plant growth by influencing membrane permeability through changes in aquaporin expression or activity. This could be validated using osmotic potential measurements or fluorescence assays targeting AQPs. Additionally, reactive species such as H_2O_2 from PAW could modulate gibberellin/abscisic acid balances via redox signaling pathways known to affect seed germination processes. Low levels of RONS help to enhance seed germination, and high concentrations will lead to increased risks of oxidative stress in seeds. The subject needs an independent study on malondialdehyde (MDA) to indicate lipid peroxidation due to excessive ROS exposure [24]. According to Table 1 (Eqs. (2) and (3)), this impact explains why water exposed to plasma has a lower pH. NO_2^- could either oxidize to NO_3^- (Eq. (4)) or react with H_2O_2 to generate the unstable intermediate peroxynitrous acid (ONOOH) (Eq. (5)), which then decomposes to NO_3^- or $\cdot NO_2$ and $\cdot OH$ radicals (Eq. (6) & Eq. (7)) under an acidic environment [25,26].

$$\cdot OH + \cdot OH \rightarrow H_2O_2 \quad (1)$$

$$NO_2(aq) + NO_2(aq) + H_2O \rightarrow NO_2^- + NO_3^- + 2H+ \quad (2)$$

$$NO\ (aq) + NO_2(aq) + H_2O \rightarrow 2NO_2^- + 2H+ \quad (3)$$

$$3NO_2^- + 4H+ + H_2O \rightarrow 2NO + NO_3^- + 3H_2O+ \quad (4)$$

$$NO_2^- + H_2O_2 + H+ \rightarrow O{=}NOOH + H_2O \quad (5)$$

$$O{=}NOOH \rightarrow \cdot NO_2 + \cdot OH \quad (6)$$

$$O{=}NOOH \rightarrow NO_3^- + H+ \quad (7)$$

Fig. 4 displays the chemical pathways used to deliver RONS by the air jet plasma into the solution. These pathways include generating and transporting ions and electrons from the plasma to the liquid and their solution, which results from the interaction between gas-phase plasmas and liquids. The reactivity transfer primarily occurs at the gas–liquid interface, involving several physical and chemical mechanisms, such as particle collisions, mass transfer, and photolysis by the absorbed UV photons and complex chemical processes [28,29].

Typically, it starts with the reactive species of an atmospheric plasma in the gas phase, where a portion of its species are transported to the plasma liquid interface, where they cross the interface and react with the liquid molecules. Notably, the gas phase radicals and molecules play a significant role in forming countless active species, such as H_2O_2, NO_2-, NO_3-, $ONOO-$, and ONOOH in aqueous solutions.

From Table 3, the wheat seeds soaked in PAW had better sprouting potential than those soaked in DW, where an estimate was conducted of the average root length of the seeds taken on day one of germination. The seeds soaked with PAW showed healthy root germination, where the average lengths of the rootlets were 7, 8.6, 8, 7.5, and 3.5 cm for S1, S2, S3, S4, and the control, respectively. The rising concentrations of reactive RONS, which stay stable in PAW for longer because of their mutual reactions

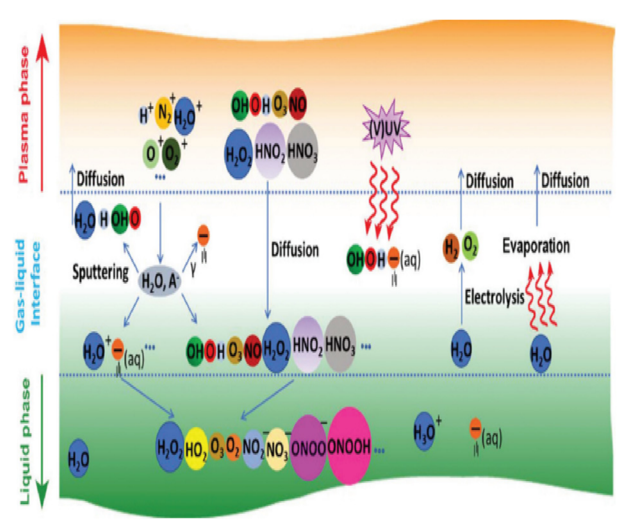

Fig. 4. The Possible mechanisms of delivery of reactive oxygen and nitrogen species (RONS) generated in the gas phase into a solution [27].

in acidic environments, maybe the source of the growth parameters' rise (Eqs. (4) and (5)). These include NO_3^-, H_2O_2, NO, and ·OH, which could stimulate molecular signaling to end physiological dormancy. They break dormancy by inducing activities like gibberellin synthesis and the degradation of abscisic acid [30].

PAW treatments impact internal transformation, imbibition, and seed water content [31,32]. At the end of the germination period, the average root length and vegetable part of seeds soaked with the treated water were significantly greater than those soaked only with DW (control), as indicated in Table 3. This was done to determine the maximum effect of the PAW on seeds.

While the reactive species have been suggested as a safe fertilizer for seeds, seed germination was linked to the ratios of the reactive species (i.e., RONS). The RONS produced in PAW may demonstrate positive signaling interference with seed dormancy and, in this way, stimulate the germination process and improve the germination profile. Several authors have stated that nitrate compounds are primarily absorbed through the roots, whether used in PAW solution or to water the plants directly. These are known to enhance growth and act as plant growth hormones. The sum of the seed properties that determine the likely level of seed activity and performance during germination, such as the appearance of the root and vegetative part, is known as "seed strength."

In group S4, the wheat seeds were directly exposed to plasma and had a much better germination rate than groups S1, S2, and S3, which were soaked in PAW, as shown in Fig. 5. This is explained by the way that RONS in PAW interact with the seed surface, potentially increasing germination rate and water and nutrient absorption. The presence of aquaporins, or membrane proteins facilitating water transport, and lipid content of the membrane are necessary for the free diffusion of H_2O_2 from the PAW across the plant cell membrane [33,34]. Yet, NO_3^- transport is a slower process controlled by specific membrane transport proteins instead of simple diffusion and is dependent on concentration. Our findings demonstrated that H_2O_2 decays far more quickly than NO_3^- and NO_2^-, which is consistent with the aforementioned. Additionally, throughout germination and imbibition, the seeds demonstrated an improved ability to detoxify H_2O_2. Therefore, seed metabolism response to H_2O_2, i.e., antioxidant enzyme activity as well as cellular signaling, is what drives effects of the PAW on the germination of seed. One of the main sources of nutrients and nitrogen for seedlings is NO_3^-.

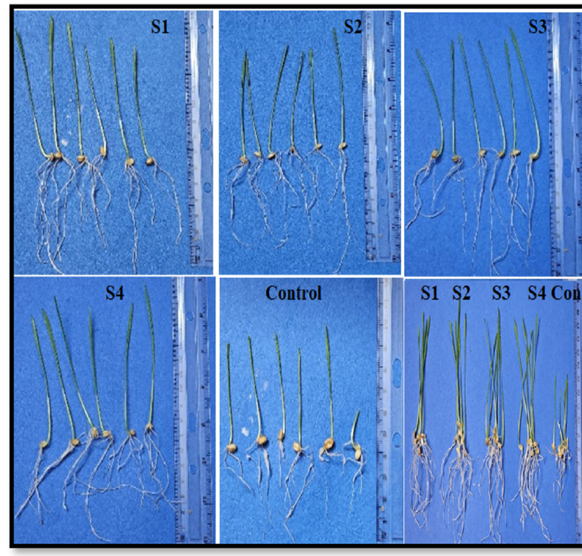

Fig. 5. Root and vegetative parts at the end of the germination period after three days.

Using eqs (8) and (9), germination rate (Rs), germination ratio (GP), and average germination time (MGT) were computed. The results are displayed in Table 4:

$$GR = \frac{NG}{NT} X\,100 \tag{8}$$

Table 4. Effect of total NO_3, NO_2, and H_2O_2 concentrations (in ppm) and pH on the germination indicators GP and GR.

Group	Reactive species (RONS) (ppm)	Germination percentage (GP)	Germination rate (GR)
S1	Total RONS = 207 NO_2 = 7 NO_3 = 100 H_20_2 = 100 PH = 2.8	98%	58
S2	Total RONS = 104 NO_2 = 4 NO_3 = 50 H_20_2 = 50 PH = 5	100%	60
S3	Total RONS = 137 NO_2 = 5 NO_3 = 66 H_20_2 = 66 PH = 4	98%	57
S4	Total RONS = 375 NO_2 = 25 NO_3 = 250 H_20_2 = 100 PH = 3	100%	58
Control	Total RONS = 0 NO_2 = 0 NO_3 = 0 H_20_2 = 0 PH = 7	91%	54.6

Where NG represents number of the germinated seeds, and NT represents total number of seeds [35].

$$GR = \sum_{i=0}^{n} \frac{Si}{Di} \quad (9)$$

Where Si represents a number of germinated seeds per count, Di represents a number of days, and n represents a number of counted days.

Petri dishes were used to cultivate seeds in vitro. A total of 91%–100% natural germination was a comparatively high germination rate for the seeds. The statistical results regarding the water treatments with plasma on wheat seeds for the seed germination rate and germination percentage rate are displayed in Fig. 6(a and b). The current findings suggest that the plasma treatment has impacted the germination rate of wheat seeds. Compared with the control, the groups that share the letter (a) had the most significant impact on the germination speed [36]. The most stimulating effect was seen in Fig. 5a when the germination rate for the PAW-soaked wheat seeds rose to 98%–100%. The germination rate was only 91% for seeds solely soaked in DW.

Data on the germination of seeds following three days of in vitro culturing are displayed in Table 2. Since most seeds germinated over the first two days and differences dropped slightly on day three, comparing Fig. 5b with the results in Table 2 reveals that germination increase has been more significant following 24 h than after three days. Acidity, oxidation-reduction potential, and electrical conductivity are all altered by plasma discharges, which

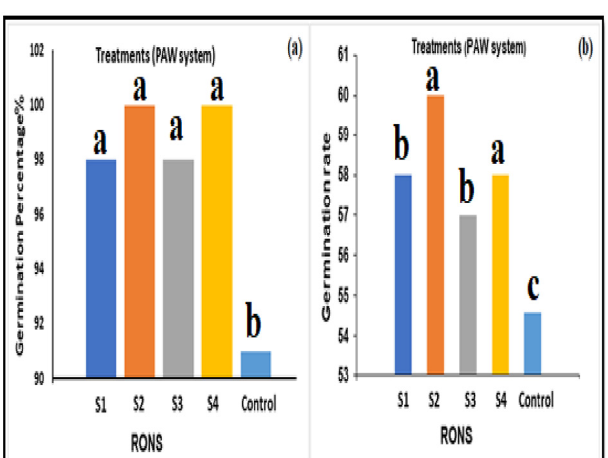

Fig. 6. (a) PAW Effect on wheat seeds' germination percentage (P < 0.01). Columns with the same letter don't differ considerably based on Duncan's multiple-range tests. (b) PAW effects on wheat seed germination rate (P < 0.01). Columns that have the same letter don't differ considerably based on Duncan's multiple-range test.

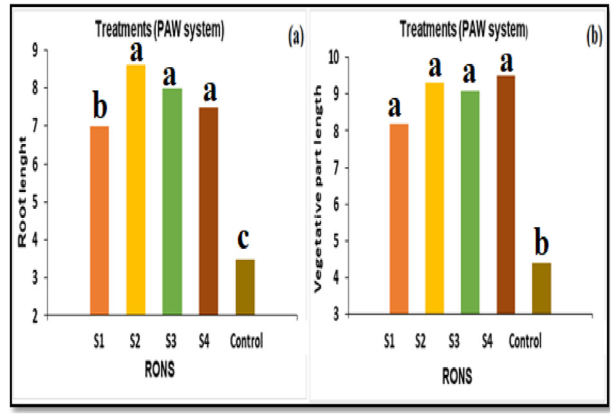

Fig. 7. (a) Effect of PAW treatments on root length of wheat seeds (P < 0.01) Columns with the same letter don't differ considerably based on Duncan's multiple range test. (b) Effects of water treatments with the use of plasma on the length of the growing part of wheat seeds (P < 0.01) Columns that have the same letter don't differ considerably based on Duncan's multiple range test.

also produce reactive oxygen and nitrogen species. As mentioned in the preceding section, these new solutions impact seed germination rate, with better seed hydration resulting in quicker germination.

The average length of the seeds' roots and vegetative part following germination is displayed in Fig. 7 a and b. Compared with the control case, wheat seeds soaked with PAW showed an improved germination rate and longer seedlings. Because of the endogenous production of NO radicals, the interaction between hydrogen peroxide and nitrate terminated seed dormancy. Increased nitrate, as well as other nitrogen ions, hydrogen peroxide, and changes in water acidity, are believed to have an impact on the enhancement of germination. Improved seed germination is linked to an increase in these variables brought on by more plasma treatment.

Despite the slight difference in RONS concentrations, the germination percentage rate, seed germination rate, the growing part length, and the root length in groups S1, S2, S3, and S4 were unaffected (Figs. 6 and 7). The seeds were treated with PAW and had statistical sufficiency compared to the control group.

6. Conclusions

A promising technique frequently used in agriculture and medicine is atmospheric pressure cold plasma. The germination of seeds and the growth of plants could be accelerated through direct plasma treatment or indirect plasma treatment that has been mediated through plasma-activated media (liquids or gases). Using a plasma jet system designed especially for such purposes, we examined the impact of PAW on wheat seeds. PAW positively accelerated the germination dynamics of wheat seeds. They soaked the wheat seeds in water. Following 20 min of exposing the water to plasma, three groups were soaked in PAW for a full day. In the fourth group, the seeds were kept in water for 24 h after being directly exposed to plasma for the same time (20 min) within the water container. The seeds in the control group were not exposed to plasma but were soaked in DW. For in vitro cultivation, the seeds were put in Petri dishes. RONS, which have biocidal and germicidal properties, enhance seed germination, and promote plant growth, are produced when the plasma jet comes into contact with water. Hydrogen peroxide, nitrates, and nitrites were the main chemical changes in the water caused by the plasma activation of DW by the plasma jet discharge (PJD). The effects of PAW on plant growth throughout the first four days were examined using wheat seeds as model farm plants. The subsequent findings were discovered:

(1) Long-living RONS, including nitrites, hydrogen peroxide, and nitrates, are rich in PAW produced through PJD.
(2) Plant growth parameters and length are marginally improved by soaking seeds in PAW.
(3) Enhancing plant growth may depend on the RONS concentration and their relative ratios, primarily hydrogen peroxide to nitrate.
(4) The comparison regarding untreated seeds soaked in DW only (control group) and seeds soaked in PAW (S1, S2, S3, and S4) revealed that the seeds of S1, S2, S3, and S4 exhibited statistical sufficiency and the best results in terms of average root length, pH decrease, and percentage of germination and rate length of the growing section.
(5) In comparison to the control group, we find that the PAW improved the vigor index, germination rate, development, and water absorption rate of wheat seedlings.
(6) Employing the water dilution approach obtained Good results and germination indicators; this condition has advantages from an economic perspective as well as from the perspective of requiring less treated water for germination.
(7) Direct exposure of wheat seeds to plasma (S4) produced the best result. The key causes of this method's results are the direct contact between the plasma and the electric discharge on the water, the larger volume of air surrounding the plasma production area, and the presence of stirrers.

The study's findings indicate that PAW has great potential for use in agriculture, from seedlings to harvest, the last stage of plant growth.

Future research Suggestions: Work on optimizing PAW treatment conditions for different crop species and exploring the long-term effects of PAW-treated plants.

Ethics Information

None.

Funding

None.

Conflicts of interest

The authors declare that they have no competing interests.

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