International Journal of Applied Agricultural Sciences
2023; 9(6): 181-190
http://www.sciencepublishinggroup.com/j/ijaas
doi: 10.11648/j.ijaas.20230906.12
ISSN: 2469-7877 (Print); ISSN: 2469-7885 (Online)

Experimental Study on the Effect of Low-Temperature Air Plasma Activated Water on Seed Germination and Seedling Growth of Maize

Zifeng Wang, Tianyi Ren, Minqi Zhou, Shuo Chen, Dexin Fu, Si Qin*

School of Electrical Engineering, Guangxi University, Nanning, China

Email address:

2112392096@st.gxu.edu.cn (Zifeng Wang), 2102010853@st.gxu.edu.cn (Tianyi Ren), 2112392127@st.gxu.edu.cn (Minqi Zhou), 2112392008@st.gxu.edu.cn (Shuo Chen), 2212392021@st.gxu.edu.cn (Dexin Fu), qinsi@gxu.edu.cn (Si Qin)

*Corresponding author

To cite this article:

Zifeng Wang, Tianyi Ren, Minqi Zhou, Shuo Chen, Dexin Fu, Si Qin. Experimental Study on the Effect of Low-Temperature Air Plasma Activated Water on Seed Germination and Seedling Growth of Maize. *International Journal of Applied Agricultural Sciences*. Vol. 9, No. 6, 2023, pp. 181-190. doi: 10.11648/j.ijaas.20230906.12

Received: March 28, 2023; **Accepted**: March 20, 2023; **Published**: November 21, 2023

Abstract: Plasma-activated water (PAW), which is produced by introducing non-thermal plasma into deionized water, contains variety of active substances such as reactive oxygen and reactive nitrogen species. These active substances in PAW exhibit sterilizing properties and facilitates intracellular chemical reactions, which holds the potential to enhance plant growth. This study aims to investigate the effects of PAW treatment on the process of maize seed germination and seedling growth. A gradient of different excitation voltages (10 kV, 14 kV, 18kV) and different treatment times (1min, 3min, 5min) was set to ionize the air to produce air-plasma, which was used to prepare the PAWs subsequently. The resulting PAWs were used to irrigate the maize seeds and seedlings every day. The effects on seed germination rate, germination potential, germination index, as well as on the chlorophyll content, peroxidase (POD) activity, and malondialdehyde (MDA) content in seedlings were examined. Treatment with PAW promoted seed germination and seedling growth, with the germination rate, germination potential, and germination index of the seeds increased by as much as 19.71%, 50.45% and 21.22% respectively. The chlorophyll and POD content in the seedlings also increased by 31.68% and 23.09% respectively. In addition, the MDA content decreased by 15.11% as compared with the control group (CK). The experimental results were subjected to significant variance analysis using the Duncan method.

Keywords: Plasma-Activated Water, Maize, Seed Germination, Chlorophyll, Peroxidase, Malondialdehyde

1. The Introduction

Plasma refers to an ionized gas consisting of active particles, such as free electrons and charged ions, which are in an ionized state. As a whole, plasma exhibits electrical neutrality and is considered the fourth state of matter. Common methods for generating plasma include dielectric barrier discharge, atmospheric pressure plasma jet, glow discharge, and corona discharge [1]. When these discharge processes occur in water, various types of active particles and free radicals are produced, including Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS). The concentrations of these species depend on the type of discharge, treatment conditions, and gas composition [2]. The resulting solution is known as PAW, which retains its strong activity even after long-term storage [3].

Due to the ability of ROS, RNS, and other active particles to promote the synthesis of plant growth hormones, enhance seed vigor, improve its resistance and help maintain cellular homeostasis [4], PAW has significant potential for increasing food production. In recent years, the application of PAW in the field of agriculture has gradually become a research focus both domestically and internationally [5].

A study was conducted wherein barley and mustard seeds were subjected to treatment with PAW for varying durations. The investigation revealed that PAW preparations with treatment durations of 5 minutes or 10 minutes exhibited the most significant impact on seed germination rate, germination

potential, and vigor index [6]. Subsequent to the foliar application of PAW onto maize leaves, a periodic observation conducted at 7-day intervals revealed a marked increase in nitrogen content within the plant biomass. However, a discernible downward trend was observed in the chlorophyll content of the leaves [7]. Upon irrigating tomato seedlings with PAW preparations crafted with treatment durations of 15 minutes, 30 minutes, and 60 minutes, it was observed that the levels of growth hormones within the seedlings were uniformly elevated. Notably, the treatment involving a 30-minute duration exhibited the most favorable outcomes in this regard [8]. Chlorophyll is ubiquitously present within green plant tissues, and its content is intricately linked to photosynthetic activity and nutritional status of plants, serving as a crucial indicator of their growth conditions. Research has demonstrated that PAW treatment can elevate the chlorophyll content in seeds [9]. Following PAW treatment of freshly cut kiwifruit, an augmentation in the content of intracellular POD was observed, POD primarily functions in the detoxification of hazardous compounds generated during cellular redox reactions through hydrolysis [10]. The application of PAW has been demonstrated to diminish the concentration of MDA within fresh goji berries. MDA content stands as a common metric for assessing plant anti-aging properties, as it reflects the extent of lipid peroxidation in cellular membranes [11]. Green mung bean sprouts were subjected to irrigation with PAW preparations generated with treatment durations of 15 seconds, 30 seconds, 60 seconds, and 90 seconds. It was observed that the sprouting rate was highest with a treatment duration of 15 seconds. Moreover, as the treatment duration increased, there was a consistent decline in the sprouting rate [12]. Distinct treatment durations of PAW were applied to Agaricus bisporus, revealing that a treatment duration of 20 minutes exhibited the most pronounced effects on the organism. Conversely, for Eruca sativa leaves treated with varying PAW treatment durations, it was observed that a duration between 2 to 5 minutes led to the greatest reduction in bacterial count, while causing the least alteration in nutritional parameters [13]. Therefore, it can be concluded that different seeds require different optimal treatment conditions, which need to be determined experimentally and cannot be generalized [14].

This study aims to investigate the effects of PAW treatment on maize seeds, which is a monocotyledonous herbaceous plant in the Poaceae family and one of the most important cereal crops globally, Its total production is only surpassed by rice and wheat [15]. The study evaluated the germination rate, germination potential, germination index, as well as the content of chlorophyll, POD, and MDA in maize seedlings treated with PAW prepared under different excitation voltages and treatment times. The pH values of each PAW group were determined, and the effect of different treatment conditions of PAW on maize seed germination and seedling growth was analyzed. The aim is to provide data support and valuable references for increasing maize production.

2. Materials and Methods

2.1. Experimental Equipment

The apparatus for generating PAW is shown (Figure 1), which consists of a plasma generation device, a high-voltage AC power supply, an air collector, and a measurement device. The PAW used in this study was prepared using the dielectric barrier discharge method, which is advantageous due to its simplicity and ease of preparation [16]. The plasma generation device is composed of a high-voltage electrode, a dielectric tube, a treatment tube, and a ground electrode. The high-voltage electrode is a tungsten needle with a diameter of 2 mm, and the dielectric tube is made of quartz with an inner diameter of 6 mm, an outer diameter of 8 mm, and a length of 135 mm. The treatment tube is a 30 mL glass tube with copper foil attached to the bottom, which is connected to the ground electrode. The plasma excitation region is located in the annular gap between the tungsten needle and the quartz dielectric tube. The high-voltage AC power supply, generated by a low-voltage DC power supply (DP605B, Shenzhen Mastech Electronics Co., Ltd.) through a high-frequency inverter, provides excitation voltages ranging from 0 to 25 kV. The air collector consists of a brushless diaphragm pump (D50H-42H, Chengdu Hailin Technology Co., Ltd.) and a gas flow meter, supplying air as the discharge gas medium and controlling the gas flow rate at 5 L·min-1. The measurement device includes a high-voltage probe (P6015A, Tektronix, Inc.), a current probe (H-FCT-200, Shanghai Pinyan Testing and Control Technology Co., Ltd.), and an oscilloscope (TDS2024C, Tektronix, Inc.), which is used to monitor the real-time voltage and current waveforms of the reaction device.

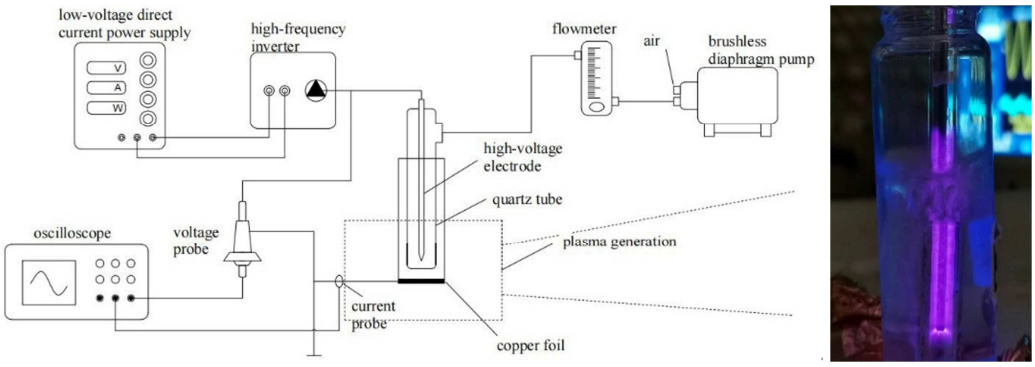


Figure 1. *Plasma-activated water reactor schematic.*

2.2. Experimental Method

PAW was prepared from deionized water using the plasma generation apparatus. Gradient excitation voltages of 10 kV, 14 kV, and 18 kV were set, and treatment times of 1 min, 3 min, and 5 min were employed. The effects of PAW prepared under different excitation conditions on seed germination rate were analyzed, and the pH values of each treatment group were measured to observe their trends.

Uniform and plump maize seeds were selected for the PAW germination experiment. Different groups of PAW were irrigated every 2 days, and the number of germinated seeds was recorded daily. After the germination period, data were analyzed and summarized. Each treatment was independently replicated three times.

Naturally germinated maize seedlings were selected for the PAW seedling cultivation experiment. The seeds were sown in seedling trays, and different groups of PAW were irrigated every day. At the end of the growth period, the chlorophyll, POD, and MDA content in the true leaves of the seedlings were measured. Each treatment was independently replicated three times.

Before conducting the germination experiment, the culture dishes were sterilized by placing them in a high-pressure sterilization pot (SX-700, Tomy Digital Biology, Japan). After the seeds were soaked and swollen in the PAW, they were placed in culture dishes lined with two layers of sterile filter paper. Thirty seeds were sown in each culture dish. Deionized water was obtained from a water purification system (WP-UP-WF-30, Waters) and treated to prepare PAW according to the different experimental conditions. The pH values of each group of PAW were measured using a pH meter (ST3100, Changzhou Aohaus Instrument Co, Ltd).

Every 2 days, 5 mL of different groups of PAW were irrigated onto the culture dishes (Figure 2). The dishes were placed in a biochemical incubator (LRH-250A, Shaoguan Taihong Medical Equipment Co, Ltd) and incubated in a light-avoiding environment at 25°C for 7 days. Seed germination was observed daily, and the number of germinated seeds was recorded. Finally, the data were summarized and analyzed.

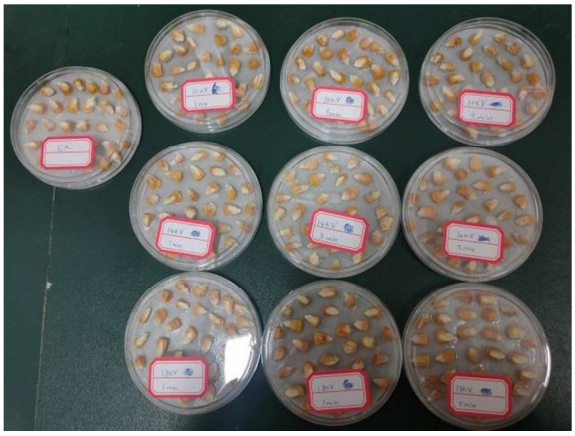


Figure 2. *Seed germination experiment conducted in culture dishes.*

Prior to the seedling cultivation experiment, the seedling trays were sterilized by disinfection. In each cell of the tray, a soil mixture consisting of 120 mg of garden soil and nutrient soil at a ratio of 1:1 was added. The seeds were placed in their respective cells. Every day, 15 mL of different groups of PAW were irrigated onto the seedlings (Figure 3 a, b). After 7 days, healthy seedlings from each group were selected, and the middle portion was removed, leaving only the cotyledons. The chlorophyll, MDA, and POD contents of the cotyledons were measured, and the data were recorded and analyzed.

(a) (b)

Figure 3. *Seedling cultivation experiment conducted in seedling trays, (a) is the CK after piped water irrigation, (b) group is the experimental group after PAW irrigation.*

2.3. Measurement Method

Seed germination was assessed based on the criterion of the embryonic shoot reaching 50% of the seed length [17], as demonstrated in Figure 4.

Figure 4. *Criterion for determining maize seed germination.*

The seed germination rate, germination index, and the calculation formula for the germination index are described as follows:

$$Germination\ rate(\%) = (N_t / N_{TS}) \times 100\% \quad (1)$$

$$Germination\ Potential(\%) = (N_{Dn} / N_{TS}) \times 100\% \quad (2)$$

$$Germination\ Index(GI) = \sum (N_{Dn} / D_n) \quad (3)$$

N_t represents the total number of germinated seeds within t days of cultivation. N_{TS} is the total number of seeds in the culture dish. N_{Dn} represents the number of germinated seeds on the nth day, where D_n denotes the germination day.

Chlorophyll is a crucial pigment in plant cells for photosynthesis and is easily soluble in organic solvents such as ethanol and acetone. It mainly consists of chlorophyll a and chlorophyll b. Chlorophyll a and chlorophyll b exhibit

maximum absorption at wavelengths of 645 nm and 663 nm, respectively [18]. For leaf sample preparation, the plant leaves under test were washed with distilled water, dried to remove surface moisture, and then the leaf lamina without the midrib was collected. Each group weighed 0.1 g of the sample, pre-cooled it with liquid nitrogen in a mortar, added 10 mL of anhydrous ethanol and acetone reagent in a 1:2 ratio, ground the mixture, transferred it to a 10 mL volumetric flask, wraped it with aluminum foil, and stored it in a light-avoiding environment for 3 hours. Subsequently, a 1mL aliquot of the extract was transferred to a glass cuvette, and the absorbance values at wavelengths of 663 nm (A_{663}) and 645 nm (A_{645}) were measured using a spectrophotometer (UV-1800, Shimadzu Corporation).

$$chlorophyll\ a\ content\left(mg/g\right) = 0.01\left(12.7A_{663} - 2.69A_{645}\right) \div W \tag{4}$$

$$chlorophyll\ b\ content\left(mg/g\right) = 0.01\left(22.9A_{645} - 4.68A_{663}\right) \div W \tag{5}$$

$$total\ chlorophyll\ content\left(mg/g\right) = 0.01\left(20.21A_{645} + 8.02A_{663}\right) \div W \tag{6}$$

W represents the sample mass [19].

POD is widely present in animals, plants, microorganisms, and cultured cells. It can catalyze the oxidation of phenolic and amine compounds by hydrogen peroxide (H_2O_2) and has a dual role in eliminating the toxicity of H_2O_2 as well as phenolic and amine compounds [20]. POD catalyzes the oxidation of specific substrates by H_2O_2, which exhibits characteristic light absorption at 470 nm. For sample preparation, each group weighed 0.1 g of the sample and mixed it with the reagent. The mixture was placed in an ice bath and centrifuged at 8000 rpm for 10 min at a temperature of 4°C using a benchtop centrifuge (Sigma 3K15, Sigma Laboratory Centrifuge Company, Germany). The supernatant was collected and transferred to a 1mL glass cuvette. The absorbance at 470 nm was measured using a spectrophotometer. The absorbance value at 30s was recorded as *A1*, and the absorbance value at 90s was recorded as *A2*. The difference in absorbance was calculated as $\Delta A = A2 - A1$.

$$POD\ content\left(U/g\right) = 7133\Delta A \div W \tag{7}$$

MDA is one of the major products of lipid peroxidation in plant cell membranes, which can be used to indicate membrane damage [21]. MDA can react with Thiobarbituric Acid (TBA) under acidic and high-temperature conditions to form a colored trimethine complex (3,5,5-trimethyl-2,4-dioxoimidazolidine) [22]. For sample preparation, each group weighed 0.1g of the sample and mixed it with the measurement reagent. The mixture was subjected to an ice bath and centrifuged at 8000 rpm for 10 min at a temperature of 4°C using a centrifuge. Subsequently, it was placed in a thermostatic water bath (JKXZ06-20B, Changzhou Nuoji Instrument Co., Ltd.) and kept for 1 hour. After cooling, the mixture was centrifuged again at 10000 rpm for 10 min at room temperature using a centrifuge. Finally, the supernatant was collected and transferred to a 1 mL glass cuvette. The absorbance at 532 nm and 600 nm was measured, and the following calculations were performed:

$$\Delta A_{532} = A_{532}measurement - A_{532}blank \tag{8}$$

$$\Delta A_{600} = A_{600}measurement - A_{600}blank \tag{9}$$

$$\Delta A = \Delta A_{532} - \Delta A_{600} \tag{10}$$

$$Malondialdehyde\ content\ \left(nmol/g\right) = 32.258\Delta A \div W \tag{11}$$

2.4. Data Analysis and Processing

The data were processed using EXCEL 2016, and graphed using Origin 2021. Statistical analysis was performed using SPSS 26.0 software. One-way analysis of variance (ANOVA) and Duncan's post hoc test were conducted to determine the significance of differences in the data. The results are presented as mean values ± standard error of the mean.

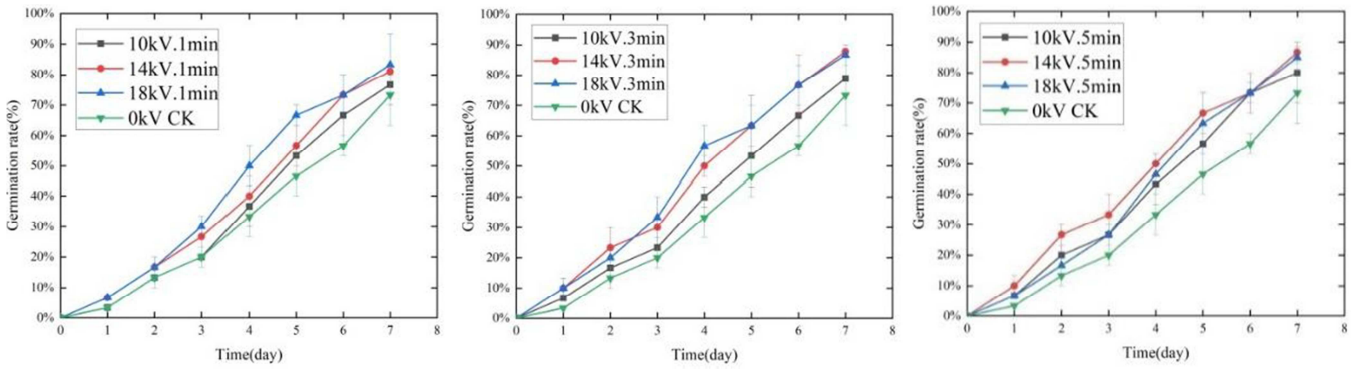
3. Results and Discussion

3.1. Effect of PAW Treatment on Seed Germination

After treatment with PAW, the germination rate, germination vigor, and germination index of maize seeds showed improvement compared to the CK. However, the effects varied among different treatment groups. The following Figure depicts the germination rates of experimental group seeds and CK seeds throughout the germination period for the same treatment duration.

It can be demonstrated that at a treatment duration of 1 min, the germination rate consistently increased with the rise in stimulation voltage (Figure 5(a)). Among the experimental groups, the one treated with 18 kV voltage exhibited the highest germination rate, with rates of 76.67%, 77.78%, and 83.33% for the three groups, respectively. The CK treated with 0 kV voltage had a germination rate of 73.33%. Although all three groups had higher germination rates compared to the CK throughout the germination period, the differences among the three groups were not significant. This lack of distinction may be attributed to the relatively short treatment duration, resulting in a lower content of active components in the PAW. For the experimental group with a treatment duration of 3 min, the germination rate initially increased and then reached a plateau with increasing voltage (Figure 5(b)). The germination rates for the three groups were 78.89%, 87.77%, and 86.67% respectively. Both the 14 kV voltage and 18 kV voltage experimental groups demonstrated significant improvement in germination compared to the 10 kV voltage group. However, there was no

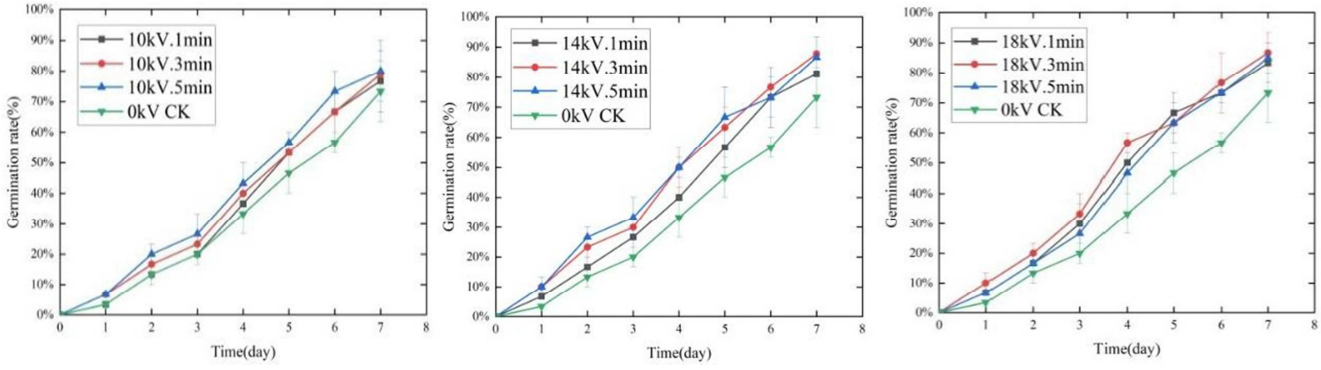
significant difference between the 14 kV voltage and 18 kV voltage groups. We speculate that the active component content in the PAW may have reached saturation when the stimulation voltage reached approximately 14 kV, thereby further increasing the voltage did not enhance the active component content in the PAW. It can be observed that at a treatment duration of 5 min, the germination rate initially increased and then decreased with increasing stimulation voltage (Figure 5(c)). Among the groups, the one treated with 14 kV voltage exhibited the highest germination rate, with rates of 80%, 87.78%, and 81.11% for the three groups, respectively. As the stimulation voltage increased, the environmental temperature also rose. Moreover, higher voltage led to greater fluctuations in temperature and pH values. Elevated temperature and acidic conditions inhibit seed growth. Hence, the germination rate of the 18 kV voltage group was lower than that of the 14 kV voltage group, possibly due to the adverse effects of higher temperature and acidity.



(a) (b) (c)

Figure 5. *(a), (b), and (c) represent the germination rates of different groups at treatment times of 1 min, 3 min, and 5 min, throughout the germination period.*

The effect of PAW prepared under different treatment time conditions on the germination rate of maize seeds varies. As shown in the following Figure 6, it presents the germination rates of the experimental groups with different treatment times and the CK seeds within the germination period, under the same excitation voltage.



(a) (b) (c)

Figure 6. *Germination rates of different groups within the germination period were recorded for excitation voltages of 10 kV, 14 kV, and 18 kV, denoted as (a), (b), and (c).*

It can be observed that the germination rate of the 10 kV voltage group shows a continuous increase with time. The germination rates of the 1min, 3min, and 5min time groups are 76.67%, 78.89%, and 80%, respectively (Figure 6(a)). Although the germination rates of all three groups are higher than that of the CK during the germination period, the differences are not significant. This could be attributed to the experimental setup where the plasma is in a recently excited state at an excitation voltage of 10 kV, resulting in the unsaturation of active components in the PAW. the 14 kV voltage group exhibits an initial increase followed by a plateau in the germination rate with increasing time (Figure 6(b)). The germination rates of the three groups are 77.78%, 87.77%, and 87.77%, respectively. It is evident that all these groups demonstrate good germination effects, indicating that the PAW prepared at an excitation voltage of 14 kV is suitable for promoting seed germination. Moreover, the germination rates of the 3 min and 5 min time groups are quite similar, suggesting that the active components in the PAW might have reached saturation at a treatment time of 3 min. Therefore, further extension of the treatment time does not significantly enhance seed germination. It can be observed that the germination rate of the 18 kV voltage group initially increases and then decreases with increasing

treatment time. The germination rates of the three groups are 83.33%, 86.67%, and 81.11%, respectively (Figure 6(c)). Among them, the 3 min experimental group shows the best performance. During the experiment, it was noticed that the 18 kV voltage group experiences greater temperature fluctuations compared to the 10 kV and 14 kV voltage groups. This group also exhibits higher temperature and lower pH. However, high temperature and acidic conditions are unfavorable for seed growth, which could be one of the reasons why the germination rate of the 5 min experimental group is lower than that of the 3 min experimental group.

3.2. Brief Summary

Table 1. *Effect of PAW with Different Treatment Conditions on Maize Seed Germination.*

Group	Excitation voltage/kV	Processing time/min	Germination rate	Germination vigor	Germination index
CK	0	0	0.733±0.033 c	0.111±0.067 d	2.615±1.945 d
1	10	1	0.767±0.067 c	0.122±0.083 c	2.650±1.825 d
2	10	3	0.789±0.033 b	0.144±0.100 bc	2.705±2.130 cd
3	10	5	0.800±0.100 b	0.150±0.083 bc	2.730±1.675 cd
4	14	1	0.811±0.067 bc	0.133±0.117 c	2.775±1.940 c
5	14	3	0.878±0.033 a	0.167±0.083 a	3.170±2.130 a
6	14	5	0.867±0.133 ab	0.167±0.033 a	3.030±1.840 b
7	18	1	0.833±0.067 b	0.144±0.050 bc	2.815±1.815 c
8	18	3	0.867±0.100 ab	0.155±0.047 b	3.025±1.865 b
9	18	5	0.850±0.033 b	0.150±0.083 bc	2.970±1.970 bc

Each value is mean of 3 replicates ± standard error of means. Different lower-case-letters in the same column indicate significant difference at $P \leq 0.05$.

(a) (b)

(c) (d)

Figure 7. *Germination specimens of experimental groups (a), (b), (c), and (d) at voltages of 10 kV, 14 kV, 18 kV, and 0 kV CK respectively, are presented in the upper Figure From left to right, each group corresponds to the experimental duration of 1 min, 3 min, and 5 min.*

Figure 7 showed the germination photographs of all experimental groups after the germination period. Overall, when the excitation voltage is 14 kV and the treatment time is 3 min, the germination rate of the seeds is the highest, and the difference from the CK is the most significant. The 10 kV voltage group showed an increase in germination rate of

4.55%, 7.58%, and 9.10% with increasing treatment time, and the 5 min experimental group exhibited a significant difference from CK. The 14 kV voltage group showed improvements of 6.07%, 19.71%, and 18.19% in germination rate, and the 3 min and 5 min time groups had a significant difference from CK. The 18 kV voltage group showed increases of 13.64%, 18.19%, and 10.61% in germination rate, and the 3 min time group differed significantly from CK. the germination rates of the experimental groups are higher compared to the CK after PAW treatment (Table 1). However, there are differences among the different groups. For the experimental groups with different excitation voltages, as the treatment time increases, the changes in seed germination rate exhibit different trends. Higher excitation voltages and longer treatment times result in better seed germination effects. However, when the experimental variables reach a certain value, the effects will weaken. Compared to the CK, the experimental group with an excitation voltage of 14 kV and a treatment time of 3 min showed the most significant difference. The germination rate was increased by 19.71%, the germination potential was increased by 50.45%, and the germination index was increased by 21.22%.

Table 2. *pH Values of PAW Prepared under Different Treatment Conditions.*

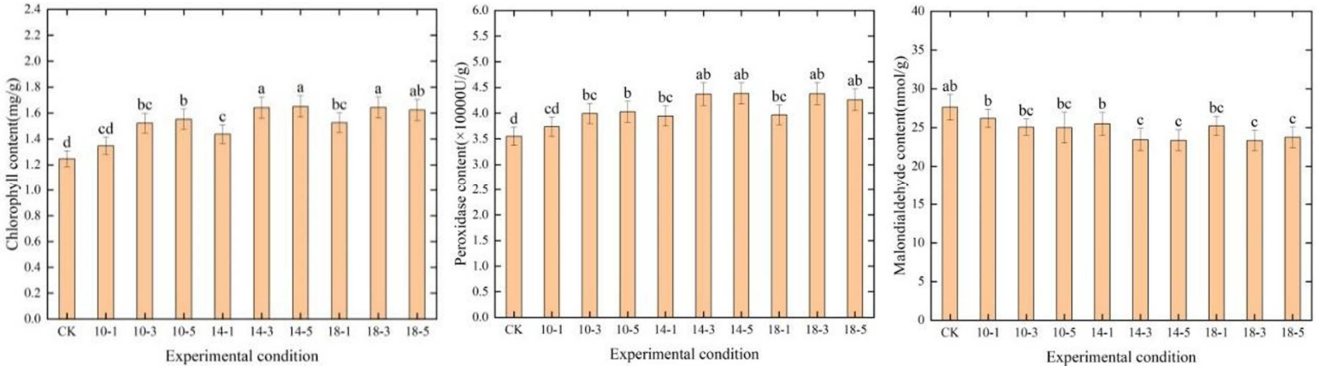
Excitation voltage/kV	**0**	**10**	**10**	**10**	**14**	**14**	**14**	**18**	**18**	**18**
Processing time/min	0	1	3	5	1	3	5	1	3	5
pH	6.93	6.16	5.56	5.35	5.77	5.43	5.24	5.58	5.36	5.06

Table 2 presents the pH values of PAW in all experimental groups. The results demonstrate a consistent decrease in the pH values of PAW as the treatment time increases or the applied voltage intensifies. This observation can be attributed to the presence of nitrite and nitrate components in the active nitrogen species of PAW. As the concentrations of these components increase, the acidity of PAW gradually intensifies. It is known that an acidic environment can inhibit plant growth. Therefore, this phenomenon can explain the trend of an initial increase followed by a decrease or stabilization in the germination rate of seeds and the levels of various hormones when higher excitation voltages are applied.

This experiment employed various treatment conditions to prepare PAW and investigated its effects on seed germination and internal hormone levels. The results revealed significant variations among the different treatments, all of which showed improvements compared to the CK. In the experiment with an excitation voltage of 10 kV, as the treatment time increased, the germination rate exhibited a continuous upward trend due to the relatively low energy density of PAW. However, the difference between this experimental group and the CK was not significant. In the experiment with an excitation voltage of 14 kV, the germination rate initially increased and then remained stable with an increase in treatment time. This trend was attributed to the higher energy density of PAW in this group, where the active components reached a saturation state after a certain period of treatment. Consequently, the difference between this group and the CK was significant. In the experiment with an excitation voltage of 18 kV, the germination rate initially increased and then decreased as the treatment time increased. We speculated that although the energy density reached saturation, the higher temperature and lower pH of PAW with prolonged treatment time created an inhibitory environment for plant growth. Furthermore, while there was a significant difference between this experimental group and the CK, the difference compared to the 14 kV experimental group was not significant. Overall, the optimal treatment conditions were an excitation voltage of 14 kV and a treatment time of 3 min.

3.3. Effect of PAW Treatment on the Growth of Maize Seedlings

After PAW treatment, the content of chlorophyll and POD in maize seedlings showed an increase compared to the CK, while the content of MDA showed a decrease. However, the effects of PAW treatment varied among different groups. The following Figure presents the content of chlorophyll, POD, and MDA in the experimental groups and the CK of maize seedlings at the end of the germination period.



(a) (b) (c)

Figure 8. *Contents of chlorophyll, POD, and MDA in the seedlings of different experimental groups is shown in Figure (a), (b), and (c), respectively. The abscissa is represented in the form of a-b to denote different treatments, where a, and b represent the excitation voltage and treatment time, respectively.*

It can be observed that the chlorophyll content of all experimental groups is higher than that of the CK. However, there were variations in chlorophyll content among different groups (Figure 8(a)). When the excitation voltage was constant, an increasing trend followed by stabilization was observed in the chlorophyll content with increasing treatment time. Overall, the experimental group with an excitation voltage of 14 kV and a treatment time of 5 min exhibited the best effect, with a 32.40% increase in chlorophyll content, showing the most significant difference compared to CK. The second best results were seen in the experimental groups with an excitation voltage of 14 kV and a treatment time of 3 min, as well as an excitation voltage of 18 kV and a treatment time of 5 min, both of which showed a 31.68% increase in chlorophyll content. The experimental groups with treatment times of 3 min and 5 min under different voltages also showed significant differences compared to CK, and they were significantly different from the 1 min experimental groups within the same excitation voltage group.

It's showed that the content of POD in maize seedlings, after treatment with PAW, is higher than that of CK. However, there were differences in POD content among different groups (Figure 8(b)). When the excitation voltage is the same, an increasing trend followed by stabilization is observed in the POD content with increasing treatment time. The experimental group with an excitation voltage of 18 kV and a treatment time of 5 min exhibited the best effect, with a 23.68% increase in POD content, showing the most significant difference compared to CK. The second best results were seen in the experimental group with an excitation voltage of 14 kV and a treatment time of 5 min, which showed a 23.45% increase in POD content. Comparing the experimental groups with treatment times of 3 min and 5 min under the three excitation voltages, no significant difference was observed, and they were significantly different from the respective 1min experimental groups. The MDA content of all experimental groups decreases compared to CK after treatment with PAW. The magnitude of the decrease varies among different groups (Figure 8(c)). Under the same excitation voltage, a decreasing trend followed by stabilization was observed in the MDA content with increasing treatment time. The experimental group with the treatment condition of an excitation voltage of 18 kV and a treatment time of 5 min showed the best effect, with a 15.57% decrease in MDA content compared to CK, showing the most significant difference. The second best effect is observed in the experimental group with an excitation voltage of 18 kV and a treatment time of 3 min, which showed a 15.44% decrease in MDA content. By comparing the data from the seedling cultivation experiment, it can be concluded that only the four experimental groups with excitation voltages of 14 kV and 18 kV, and treatment times of 3 min and 5 min, showed significant differences compared to CK. They exhibited decreases in MDA content of 15.11%, 15.42%, 15.44%, and 15.57%, respectively. The differences between the remaining treatment conditions and CK were not significant.

Table 3. *Effect of PAW with Different Treatment Conditions on Hormone Content in Seedlings.*

Group	Excitation voltage/kV	Processing time/min	Chlorophyll/(mg/g)	POD /($\times10^4$U/g)	MDA /(nmol/g)
CK	0	0	1.247±0.126 d	3.552±0.342 d	27.631±1.423 ab
1	10	1	1.347±0.128 cd	3.737±0.326 cd	26.183±1.265 b
2	10	3	1.523±0.146 bc	3.994±0.385 bc	25.056±1.337 bc
3	10	5	1.553±0.153 b	4.029±0.359 b	24.993±1.358 bc
4	14	1	1.437±0.143 c	3.946±0.337 bc	25.485±1.269 b
5	14	3	1.642±0.156 a	4.372±0.411 ab	23.457±1.382 c
6	14	5	1.651±0.162 a	4.385±0.394 ab	23.369±1.297 c
7	18	1	1.525±0.148 bc	3.967±0.406 bc	25.236±1.373 bc
8	18	3	1.644±0.154 a	4.382±0.442 ab	23.337±1.309 c
9	18	5	1.623±0.156 ab	4.263±0.425 ab	23.751±1.365 c

Each value is mean of 3 replicates ± standard error of means. Different lower-case-letters in the same column indicate significant difference at $P \leq 0.05$.

The data indicate that the hormone levels in seedlings vary to different extents compared to the CK after PAW treatment. With one treatment variable held constant, the levels of chlorophyll and POD enzyme in seedlings exhibited an initial increase followed by stabilization as the other treatment variable increases. Conversely, the content of MDA showed an initial decrease followed by stabilization (Table 3). These findings suggest that higher excitation voltage and longer treatment time yield better effects; however, the effectiveness diminishes after reaching a certain threshold. Overall, the optimal treatment conditions were observed at an excitation voltage of 14 kV and treatment times of 3 min and 5 min. The chlorophyll content increased by 31.68% and 32.40% respectively, the POD enzyme content increased by 23.09% and 23.45% respectively, and the MDA content decreased by 15.11% and 15.42% respectively in these two experimental groups. These differences were statistically significant.

In this experiment, we investigated the impact of PAW prepared under various treatment conditions on seedling hormone levels. The results revealed that after PAW treatment, all groups exhibited increased chlorophyll and POD content compared to the CK, while MDA content decreased. The optimal treatment condition was observed at a stimulation voltage of 14 kV with a treatment duration of 3 min. The elevation in POD content is likely to enhance plant respiration and photosynthesis, suggesting that it may also

promote chlorophyll synthesis and MDA reduction. Plant leaf senescence is a multifactorial and co-regulated physiological process [23]. The experimental findings indicated that PAW treatment can delay plant aging without inhibiting cellular membrane lipid oxidation. Instead, nitrites in PAW react with MDA to facilitate its decomposition [24]. The promotion of seed germination and seedling growth by PAW can be attributed to the presence of ROS and RNS. ROS maintains cellular homeostasis, while RNS decomposes harmful substances within cells, with the primary components being nitrate and nitrite ions formed by the oxidation of NO by H_2O_2 and O_3 [25]. PAW exerts a direct chemical impact on plant cell walls, thereby triggering multiple molecular signaling pathways and engaging tissue softening processes to alleviate seed dormancy blocks. This consequently advances the termination of seed dormancy ahead of schedule [26]. PAW enhances seed water absorption, promotes germination, and influences hormone production within plants [27, 28]. Furthermore, research indicated that PAW-covered soil experiences minimal changes in water evaporation and maintains a neutral pH range even with extensive use, while nitrogen content in the soil increases [29]. Thus, PAW has no adverse impact on the physicochemical properties of the soil and represents a safe, sustainable, and environmentally friendly approach with significant development prospects [30, 31].

In our forthcoming research endeavors, we intend to further optimize the preparation conditions of plasma-activated water to explore a broader array of experimental variables. Additionally, we aim to measure a greater variety of enzymes and growth hormone contents during the maize crop growth process. Furthermore, we will conduct an in-depth analysis of the active components within plasma-activated water to elucidate which components play the most crucial role in maize growth. Subsequently, we will engage in simulation experiments to validate the empirical findings.

4. Conclusion

The results of this study demonstrated that PAW treatment can improve the germination rate, germination vigor, and germination index of maize seeds, as well as the content of chlorophyll and POD enzyme during seedling growth, while reducing the content of MDA. However, the effects of PAW varied with different treatment parameters. Through the significant difference analysis using Duncan's method on the experimental data, it was found that when the excitation voltage was 14 kV and 18 kV, and the treatment time was 3 min and 5 min, the effects of PAW treatment were significantly different from the CK. However, the differences among these four groups were not significant. From the perspective of energy conservation, the optimal treatment conditions in this experiment were an excitation voltage of 14 kV and a treatment time of 3 min. At this point, the pH of PAW was 5.43, indicating weak acidity. Since the three hormones we measured have different functions in plant cells, we speculated that there is a connection between them. The increase in peroxidase enzyme activity promotes plant respiration and photosynthesis, thereby facilitating the synthesis of chlorophyll and the metabolism of MDA in plant cells. In the future, we will further optimize the experimental parameters and explore more treatment conditions to investigate their effects on seeds and their effectiveness.

Conflicts of Interest

The Authors declare that there are no competing interest.

References

[1] Guo D, Liu H, Zhou L et al. Plasma-activated water production and its application in agriculture. [J]. Journal of the Science of Food and Agriculture, 101 (12), 4891-4899 (2021).

[2] Bradu C, Kutasi K, Magureanu M et al. Reactive nitrogen species in plasma-activated water: generation, chemistry and application in agriculture. [J]. Journal of Physics D: Applied Physics 53 (22), 223001 (2020).

[3] Guo L, Xu R, Gou L et al. Mechanism of virus inactivation by cold atmospheric-pressure plasma and plasma-activated water. [J]. Applied and environmental microbiology 84 (17), e00726-18 (2018).

[4] Rathore V, Patel D, Butani S et al. Investigation of physicochemical properties of plasma activated water and its bactericidal efficacy. [J]. Plasma Chemistry and Plasma Processing 41, 871-902 (2021).

[5] Dumanović J, Nepovimova E, Natić M et al. The significance of reactive oxygen species and antioxidant defense system in plants: A concise overview. [J]. Frontiers in plant science. 11, 552969 (2021).

[6] Zambon Y, Contaldo N, Laurita R et al. Plasma activated water triggers plant defence responses. [J]. Scientific Reports. 10 (1), 19211 (2020).

[7] Guragain R P, Baniya H B, Shrestha B et al. Improvements in germination and growth of sprouts irrigated using plasma activated water (PAW). [J]. Water. 15 (4), 744 (2023).

[8] Škarpa P, Klofáč D, Krčma F et al. Effect of plasma activated water foliar application on selected growth parameters of maize (Zea mays L.). [J]. Water. 12 (12), 3545 (2020).

[9] Adhikari B, Adhikari M, Ghimire B et al. Cold atmospheric plasma-activated water irrigation induces defense hormone and gene expression in tomato seedlings. [J]. Scientific reports. 9 (1), 16080 (2019).

[10] Rathore V, Tiwari B S, Nema S K. Treatment of pea seeds with plasma activated water to enhance germination, plant growth, and plant composition. [J]. Plasma Chemistry and Plasma Processing, 1-21 (2022).

[11] Zhao Y, Chen R, Liu D et al. Effect of nonthermal plasma-activated water on quality and antioxidant activity of fresh-cut kiwifruit. [J]. IEEE Transactions on Plasma Science. 47 (11), 4811-4817 (2019).

[12] Cong K P, Li T T, Wu C E et al. Effects of plasma-activated water on overall quality of fresh goji berries during storage. [J]. Scientia Horticulturae. 293, 110650 (2022).

[13] Fan L, Liu X, Ma Y et al. Effects of plasma-activated water treatment on seed germination and growth of mung bean sprouts. [J]. Journal of Taibah University for Science. 14 (1), 823-830 (2020).

[14] Zhao Z, Wang X, Ma T. Properties of plasma-activated water with different activation time and its effects on the quality of button mushrooms (Agaricus bisporus). [J]. LWT. 147, 111633 (2021).

[15] Laurita R, Gozzi G, Tappi S et al. Effect of plasma activated water (PAW) on rocket leaves decontamination and nutritional value. [J]. Innovative Food Science & Emerging Technologies. 73, 102805 (2021).

[16] Ruan Z, Wang X, Liu Y et al. Corn [M] // Integrated Processing Technologies for Food and Agricultural By-Products. Academic Press, 59-72 (2019).

[17] Jin Y S, Cho C, Kim D et al. Mass production of plasma activated water by an atmospheric pressure plasma. [J]. Japanese Journal of Applied Physics. 59 (SH), SHHF05 (2020).

[18] Zhu Tong, Zhang Di, Tang Hongwei et al. Application of plasma activated water in seed pre-sowing treatment. [J]. Agricultural Engineering. 12 (10), 22-29 (2022).

[19] Ercoli L, Mariotti M, Masoni A et al. Relationship between nitrogen and chlorophyll content and spectral properties in maize leaves. [J]. European Journal of Agronomy. 2 (2), 113-117 (1993).

[20] Dumanović J, Nepovimova E, Natić M et al. The significance of reactive oxygen species and Bachmann L M, Miller W G. Spectrophotometry [M] // Contemporary Practice in Clinical Chemistry. Academic Press, 119-133 (2020).

[21] de Oliveira F K, Santos L O, Buffon J G. Mechanism of action, sources, and application of peroxidases. [J]. Food Research International. 143, 110266 (2021).

[22] Marnett L J. Lipid peroxidation—DNA damage by malondialdehyde. [J]. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 424 (1-2), 83-95 (1999).

[23] Valenzuela A. The biological significance of malondialdehyde determination in the assessment of tissue oxidative stress. [J]. Life sciences. 48 (4), 301-309 (1991).

[24] Chu Mengyuan, Yu Yanchong. Research progress on factors affecting plant leaf senescence, [J]. Life Sciences. 31 (2), 178-184 (2019).

[25] Ke Z, Bai Y, Yi Y et al. Why plasma-activated water treatment reduced the malonaldehyde content in muscle foods. [J]. Food Chemistry. 403, 134387 (2023).

[26] Tachibana K, Oh J S, Nakamura T. Oxidation processes of NO for production of reactive nitrogen species in plasma activated water. [J]. Journal of Physics D: Applied Physics. 53 (38), 385202 (2020).

[27] Grainge G, Nakabayashi K, Steinbrecher T et al. Molecular mechanisms of seed dormancy release by gas plasma-activated water technology. [J]. Journal of Experimental Botany. 73 (12), 4065-4078 (2022).

[28] Bafoil M, Jemmat A, Martinez Y et al. Effects of low temperature plasmas and plasma activated waters on Arabidopsis thaliana germination and growth. [J]. PloS one. 13 (4), e0195512 (2018).

[29] Porto C L, Ziuzina D, Los A et al. Plasma activated water and airborne ultrasound treatments for enhanced germination and growth of soybean. [J]. Innovative Food Science & Emerging Technologies. 49, 13-19 (2018).

[30] Rathore V, Nema S K. Optimization of process parameters to generate plasma activated water and study of physicochemical properties of plasma activated solutions at optimum condition. [J]. Journal of Applied Physics. 129 (8), 084901 (2021).

[31] Šimečková J, Krčma F, Klofáč D et al.: Influence of plasma-activated water on physical and physical–chemical soil properties. [J]. Water. 12 (9), 2357 (2020).