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Enhancing seed germination of *Abelmoschus esculentus* (L.) Moench and *Ipomoea aquatica* Forssk. through atmospheric pressure plasma-activated distilled water

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ABSTRACT

Seed priming is a pre-planting activity that has been found to enhance seed vigor and improve seed germination, particularly in species with poor or inconsistent germination rates or in deteriorated or poorly stored seeds. Several priming agents have been investigated, and one emerging priming agent is plasma-activated water (PAW). This study aimed to determine the effects of atmospheric pressure plasma (APP)-activated distilled water on the germination and seedling characteristics of *Abelmoschus esculentus* (L.) Moench and *Ipomoea aquatica* Forssk. Distilled water was treated with APP for 1, 3, and 5 minutes. Electrical conductivity (EC), pH, resistivity, total dissolved solids (TDS), oxidation-reduction potential, contact angle, temperature, and surface tension were determined. Further, germination tests were conducted to determine the effect of pre-soaking seeds in PAW on germination and seedling characteristics. As exposure time (ET) to APP increased, the pH, EC, and TDS of PAW also increased, while resistivity decreased. No definite effects were observed on the other physical parameters. *Abelmoschus esculentus* and *I. aquatica* showed a greater increase in seed area after pre-soaking in PAW for 8 h compared to the control. Further, PAW-treated seeds of both species exhibited higher percent water imbibition compared to the control. Regardless of ET, PAW-treated seeds had higher germination percentage, germination rate index, faster germination, and shorter germination time. Seedlings of PAW-treated seeds developed longer roots, heavier root and shoot biomass, and a higher seedling vigor index. These findings provide evidence that PAW is a potential seed priming treatment for *A. esculentus* and *I. aquatica*. The researchers propose that pre-soaking seeds in plasma-activated water exposed to APP for as little as one minute can significantly enhance germination rates and improve seedling traits.

Keywords: atmospheric pressure plasma jet, plasma agriculture, seed invigoration, seed technology, vegetables

INTRODUCTION

High and uniform germination is one of the primary factors that need to be considered to attain

cropping sustainability and success (Reed et al. 2022). The importance of high-quality seed germination responses contributes directly to crop production and food security (Finch-Savage and Bassel 2016).



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Therefore, pre-planting activities like seed germination and treatment, as well as the selection of seed varieties, are important factors that need to be investigated for efficient and sustainable food production (Lamichhane et al. 2018). Farmers frequently face challenges with low seed germination, which can be attributed to various factors such as improper storage conditions, unfavorable climatic influences, pathogen infestations, reduced seedling vigor, and infections caused by seed-borne pathogens (Jose et al. 2023). Low seed germination rates and diminished seedling vigor can be effectively addressed through various enhancement techniques, with seed priming emerging as a particularly promising approach (Devika et al. 2021).

Seed priming is the conditioning for seeds through controlled hydration at a level that allows activation of pregerminative metabolic activity but not sufficient to proceed to radicle emergence (Raj and Sheeja 2019). It is commonly done to hasten germination and to improve the uniformity of seedling growth (Zulfiqar 2021). Priming also improves plant tolerance to drought and other extreme conditions (Hussain et al. 2022). Multiple techniques and materials have been used as priming agents. Among the priming agents are essential oils, chemical salts, plant extracts, bacterial metabolites, and treated water or water exposed to physical processes such as radiation, sonication, and plasma, such as atmospheric plasma (Bafoil et al. 2018; Bera et al. 2022).

Plasma activated water (PAW) is currently considered an emerging priming agent. The PAW can be produced by exposing water to cold plasma discharge from an atmospheric pressure plasma (APP) jet for a specific amount of time. Exposure of water to plasma causes changes in the physical characteristics of water such as pH, electric conductivity (EC), and total dissolved solids (TDS). The interaction of plasma and water causes increased amounts of reactive oxygen and nitrogen species (RONS), which have been reported to improve germination, growth, and development of plants, increase plant tolerance to diseases, delay ripening, and promote reinvigoration, among others (Adhikari et al. 2020). Numerous studies have been conducted to examine the effects of PAW. However, in the Philippines, research on this topic remains limited, particularly in crops such as *Abelmoschus esculentus* (L.) Moench and *Ipomoea aquatica* Forssk. Since plant species exhibit varying responses to treatments, it is essential to document these differences. Therefore, this study was conducted to explore the germination of these two species, providing a foundation for future research and further investigation.

By observing and documenting the effects of PAW on germination and seedling growth in *A. esculentus* and *I. aquatica*, the results of this study can contribute to the development of newer seed invigoration techniques that may improve crop production, minimize seed losses, and promote adoption of eco-friendly technologies. Plasma technology is recognized as an eco-friendly innovation due to its avoidance of harmful chemicals and pollutants, making it a sustainable alternative for various applications. Further, plasma treated water can still be recycled without drastic changes in its physical properties (Sajib et al. 2020).

The general objective of the study was to determine the effects of APP-treated distilled water on the germination of seeds, as well as the seedling characteristics of *A. esculentus* and *I. aquatica*. Specifically, the study aimed to physically characterize distilled water treated with APP at varying exposure time (ET), to correlate different physical properties of PAW with ET, and to evaluate the effect of presoaking seeds with distilled water treated with APP at varying ET on germination and seedling characteristics.

METHODS

Preparation of Distilled Plasma-activated Water

The schematic representation of the APP device used in this study is shown in Figure 1. The electrodes were two 0.5 mm diameter tungsten (W) rods enclosed in a custom-built glass tube with a 14 mm outside diameter sealed with rubber (Figure 1). The spacing between the W rods was approximately 10 mm, and they were connected to a 10 kV, 30 mA neon light power supply. The upper part of the custom-built glass tube had two openings as gas inlets. One was sealed with a rubber stopper, while the other was connected to an air compressor that delivered 55 L min⁻¹. Distilled water (200 mL) was exposed to APP with a nozzle-to-water distance at approximately 5 cm at 0, 1, 2, 3, 4, and 5 min for the preliminary measurements and 0, 1, 3, and 5 min for the seed application.

Characterization of Distilled Plasma-activated Water

For the measurements of physical properties of distilled water treated with APP at various exposure times, three trials were conducted. Physical parameters measured included pH, temperature (T), electrical conductivity (EC), resistance, total dissolved solids (TDS), contact angle (CA), and surface tension (ST). A handheld automatic water quality meter (YY-

1010) was used to measure pH, T ($^{\circ}\text{C}$), TDS (TDS), EC ($\mu\text{S cm}^{-1}$), and R ($\text{M}\Omega$). The resolution and accuracy of pH, EC, TDS, T, and R were 0.01 ± 0.02 pH, $1 \mu\text{S cm}^{-1} \pm 2\%$ full-scale temperature, $1 \text{ ppm} \pm 2\%$ full-scale temperature, $0.1^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, and $0.1 \text{ M}\Omega \pm 1\%$ full scale, respectively. Other parameters such as CA and ST were measured using Ossila goniometer. A 1 cc syringe was used to drop PAW onto cleaned glass slides.

Preparation of Seed Treatments

The experiments were arranged in a completely randomized design (CRD) with three replications and two subsamples each for the germination test to determine seed germination and seedling characteristics. One hundred seeds were used for each subsample for a total of 2,400 seeds per species. Further, for seedling characteristics, 10 seedlings were measured per subsample, with a total of 300 seedlings per species. There were three pre-soaking treatments (APP-treated distilled water with 1-, 3- and 5- min exposure durations) and a control treatment (untreated distilled water). Distilled water was purchased commercially. Seeds of *A. esculentus* and *I. aquatica*, sourced from commercial seed stores in Quezon City, Philippines, were used in the study. Seeds of *A. esculentus* and *I. aquatica* were obtained from Condor Quality seeds last 2023.

Presoaking, Germination Tests, and Seedling Characterization

The seeds were subjected to pre-germination analysis using the method published by Adhikari et al. (2021). Ten seeds per subsample were used for each species. Seed weight was measured initially using a digital analytical balance and then seeds were soaked in treatments for 8 h. After soaking, the seeds were blot- and air-dried for 5 min. The final weights of the

seeds were determined. Percent water imbibition was calculated using the formula in Table 1. Further increase in seed area was measured using a dissecting microscope and ImageJ®, where initial (before soaking) and final (after 8 h soaking) seed areas were measured using the formula in Table 1.

A separate batch of seeds was used for the germination tests. Seeds were soaked in treatments for 12 h and air-dried for 8 h before being subjected to a germination test using the top-of-paper method (Rao et al. 2006). A day after sowing, manual counting of germinated seeds was done, characterized by radicle protrusion. Observation was continued until the 7th day of germination. Germination percentage (GP%), germination rate index (GRI), mean germination time (MGR), and germination speed (GS) were computed. The formulas for these germination parameters are shown in Table 1.

Percent germination (%) (GP). One hundred seeds per subsample for three replicates were sown in moistened paper towels placed in plastic tubs. Seeds were placed equidistantly on the moist paper towels, which were sprayed with 6-7 mL of distilled water using an atomizer during the 14-day testing period to keep the setup moist. Counting of seedlings was done daily for 14 days. A higher germination percentage indicated higher seed viability.

Germination rate index (GRI). This parameter combines both germination percentage and germination time (Tadros et al. 2011). For GRI, a higher value indicates higher vigor and more uniform, earlier germination (Bhadra et al. 2024). Daily counting of germinated seeds was performed from day 1 to day 14. Further, the higher germination rate index indicates higher seed vigor which specifically means earlier yet uniform germination.

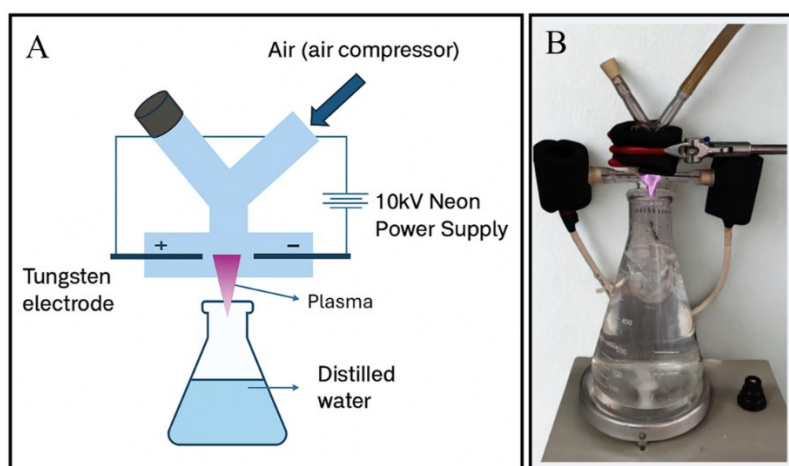


Figure 1. (A) Schematic of the atmospheric pressure plasma jet set-up (Park et al. 2013; Malapit and Baculi 2021) and (B) actual distilled water treatment.

Table 1. Summary of the formulas used in the study.

Parameter	Formula
%Imbibition	$((\text{Seed wt. } _f - \text{Seed wt. } _i) / \text{Seed wt. } _i) \times 100$
%Increase in Seed Area	$((\text{Seed area } _f - \text{Seed area } _i) / \text{Seed area } _i) \times 100$
Germination Percentage	$((\text{Total no. of seedlings}) / (\text{No. of seeds sown})) \times 100$
Germination Rate Index	$n/1 + \dots + n/14; n = \text{no. of seedlings}$
Mean Germination Time	$\sum (\text{Daily no. of germinated seeds} \times \text{no. days from beginning of test}) / (\text{Total germinated seed at final count})$
Germination Speed	$\frac{(\text{Total no. of seedlings at Day } _i / \text{Day count } _{\text{first}} + \text{Total no. of seedlings at Day } _f / \text{Day count } _{\text{final}})}{}$
Vigor Index I	$(\text{Root length} + \text{Shoot length}) \times \text{Germination percentage}$

Mean germination time (MGT). Mean germination time measured the time it took for the seed to germinate, focusing on the day at which most seeds had germinated. A lower MGT indicated a shorter time for germination (Orchard 1977).

Germination speed (GS). This parameter represented the rate of germination in terms of the total germinated seeds in a given time frame. A higher germination speed indicated faster germination (Abbasi Khalaki et al. 2019). The data from daily germination were used to calculate GS (Table 1).

Seedling growth (root and shoot length and weight, number of secondary roots). Seedling growth was measured by selecting 10 seedlings per subsample. Root and shoot lengths (cm) were measured using a ruler, while root and shoot weights (g) were obtained using an analytical balance to determine fresh weight.

Vigor Index II. Vigor Index II (VII) was calculated by determining the sum of root and shoot lengths and multiplying by germination percentage. A higher seed vigor index indicated that the seed lot was more vigorous. The VII was considered important factor providing insight into seed stress tolerance. Seed vigor pertains to uniformity of seed germination (Ranmeechai et al. 2022). Higher vigor index indicated higher seedling quality and computed using the formula (Table 1).

Statistical Analysis

The data on the physical parameters of PAW were collected in five replications with three subsamples per replication. The average of each physical parameter was plotted against the treatment (exposure time). Further, other parameters are presented in Table 1. Moreover, the mean values of different physical parameters of PAW-treated water were determined. The data on the physical parameters

of PAW were analyzed using Analysis of Variance (ANOVA) after meeting assumptions of normality and homogeneity of variance. A Kruskal-Wallis test, a non-parametric method, was employed to assess significant differences in oxidation-reduction potential, followed by Dunn's test for pairwise comparisons. Additionally, the relationship between physical parameters (that exhibited normality and homogeneity of variance) and exposure time was examined using Pearson correlation analysis and was visualized with the *ggcorrplot* package in R (Wei and Simko 2024).

Seed germination and seedling characteristics data were analyzed using Analysis of Variance (ANOVA) after meeting assumptions of normality and homogeneity of variance. Analysis was conducted through Jamovi ® software to determine whether treatment or exposure time of APP-treated distilled water had an effect on pre-germination, germination, and seedling characteristics of *A. esculentus* and *I. aquatica*. Pairwise mean comparisons were performed using the least significant difference (LSD) test at a 5% level of significance.

RESULTS

Characteristics of Plasma Activated Water (PAW)

The characteristics of PAW in terms of acidity, total dissolved solids, electric conductivity, and resistivity were plotted against the exposure time to APP, as shown in Figure 2. Specifically, pH measured the acidity and basicity of the material. Total dissolved solids, on the other hand, represented the total amount of solids dissolved in water. The solids included soluble hydrogen carbonate ions, chloride salts, and non-volatile solids, among others. Further, electric conductivity referred to the ability of the

material to pass the flow of electric current, while resistivity measured how strongly a material resisted the electric current. The data showed that the acidity increased as the exposure time of distilled water to APP was prolonged.

The pH underwent a significant reduction from 6.67 to 3.67 within just 1 minute of exposure to APP (Figure 2A). However, the rate of pH changes progressively decreased with prolonged exposure durations. In terms of electric conductivity, distilled water increased its EC as ET to APP was prolonged from $2 \mu\text{S cm}^{-1}$ to $126 \mu\text{S cm}^{-1}$ to $459.33 \mu\text{S cm}^{-1}$. This pattern was similarly observed in total dissolved solids, where TDS increased in distilled water as the exposure time to APP was prolonged (from 0 to 228.33 ppm). However, in terms of resistivity, there was a decrease in resistivity as the exposure time of distilled water to APP was extended. Correlational analysis of these characteristics was conducted to determine the relationships of these parameters across increasing exposure times (Figure 2).

Other physical parameters of distilled water treated with APP at varying exposure times are presented in Table 2. No significant differences were observed in temperature, contact angle, and surface tension of water treated or exposed to APP at varying durations. Further, significant differences were observed in the oxidation reduction potential (ORP) of PAW compared to the control. The highest ORP was observed in distilled water exposed to APP for 4 to 5

minutes (646–662 V). Specifically, there was almost a 50% increase in oxidation-reduction potential when distilled water was exposed to APP for 1 minute and a gradual increase thereafter.

Correlational Analysis of Different PAW Physical Parameters and Exposure Time to APP

Correlation analysis of four physical parameters of treated distilled water and exposure time to atmospheric pressure plasma revealed significant relationships among pH, R, EC, and TDS when correlated with exposure time (ET) to APP. A significant correlation was observed on pH, EC, TDS, and R when correlated with ET to APP. A strong negative correlation was observed between the pH of treated water and ET ($P = 0.000$, $r = -0.77$), while a moderate negative correlation was observed when R was correlated with ET. However, a very strong positive correlation was observed when EC and TDS were correlated with ET ($P = 0.000$, $r = 0.98$; $P = 0.000$, $r = 0.98$, respectively). Physical properties of APP-treated distilled water were also correlated with each other. R had a strong negative correlation with EC ($P = 0.000$, $r = -0.61$) and TDS ($P = 0.000$, $r = -0.61$). However, it had a strong positive correlation with pH ($P = 0.000$, $r = 0.74$). Moreover, a very strong negative correlation was observed when pH was correlated with EC ($P = 0.000$, $r = -0.83$) and TDS ($P = 0.000$, $r = -0.83$) (Figure 3).

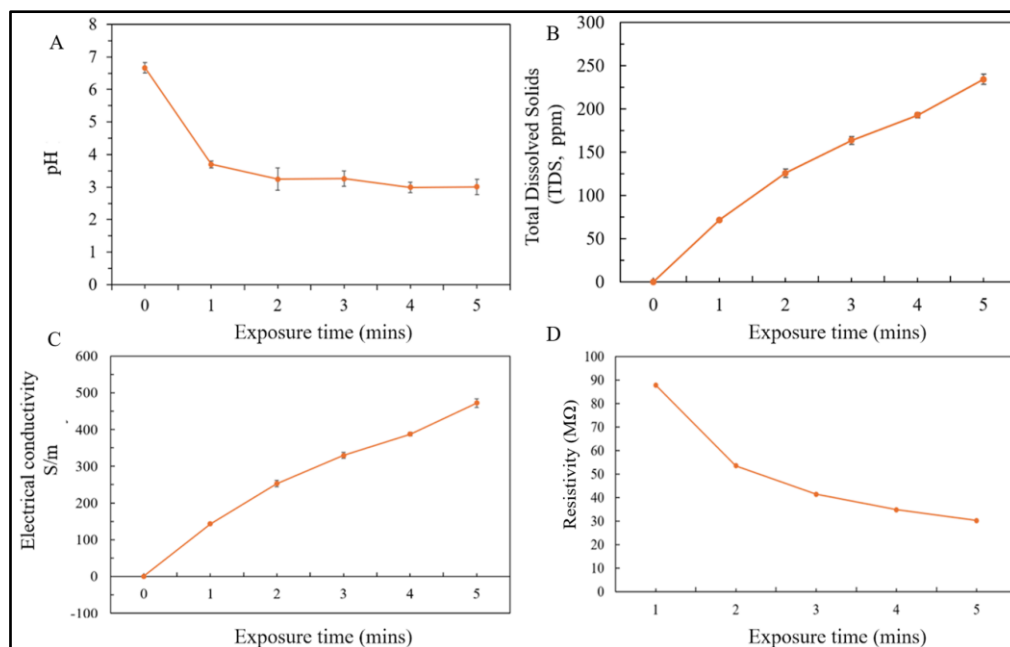


Figure 2. Physical characteristics—(A) pH, (B) total dissolved solids, (C) electric conductivity, and (D) resistivity—of plasma activated water plotted against the exposure time of distilled water to atmospheric pressure plasma.

Table 2. Other physical characteristics of distilled water treated with atmospheric pressure plasma in varying exposure times. Different superscript letters indicate the significant differences in mean values at $P < 0.05$.

Exposure Times (min)	Temperature (°C)	Oxidation-Reduction Potential (V)	Contact Angle (°)	Surface Tension
0	30.61 ± 2.35	196.33 ± 14.93 ^c	39.07 ± 0.97	299.16 ± 38.98
1	30.53 ± 2.26	382.67 ± 59.34 ^b	37.94 ± 0.96	305.03 ± 4.48
2	30.72 ± 2.12	462.00 ± 2.52 ^b	39.67 ± 0.66	296.90 ± 16.04
3	30.83 ± 2.42	569.67 ± 1.16 ^a	38.38 ± 0.94	308.12 ± 16.04
4	31.06 ± 2.43	646.00 ± 14.34 ^a	40.64 ± 0.61	304.4 ± 15.11
5	31.07 ± 2.43	662.00 ± 3.05 ^a	39.25 ± 1.01	332.12 ± 19.40
<i>P</i> value	0.977	< 0.001	0.884	0.40



Figure 3. The correlogram shows correlation coefficients for selected physical parameters pH, resistivity, electric conductivity, and total dissolved solids of distilled water treated with atmospheric pressure plasma at varying exposure times. Variables with more intense colors and larger sizes indicate stronger correlations, with blue signifying positive correlations, whereas red indicates negative correlations.

Effect of PAW on Germination, and Seedling Characteristics

Significant differences were observed in the response of *A. esculentus* seeds soaked in distilled water treated with APP at various exposure times. For *A. esculentus*, seeds treated with PAW were larger than the control regardless of ET (Figure 4). This was similar to *I. aquatica*, where PAW-treated seeds showed a higher increase in seed area compared to the control (Figure 5). Water uptake or seed water imbibition is a vital step to initiate germination. Imbibition or water uptake occurred when seeds were exposed to water. Water imbibition promoted enzyme hydration, which in turn activated enzymes. Important hydrolytic enzymes included proteases, amylases and lipase, which played a role in the breakdown of food sources in the seed (Joshi 2018). Water uptake or imbibition was observed through swelling and softening of the testa. Figure 5 showed the morphological swelling and softening of the testa across three species. As shown in Figure 1, PAW significantly increased water absorption in *I. aquatica* ($P = 0.00$) and *A. esculentus* ($P = 0.046$) when compared to the control (distilled, untreated). Moreover, similar responses were observed in seeds treated with PAW regardless of the PAW's ET to APP.

The effects of PAW treatment on seed germination are presented in Table 3. In all selected germination parameters, GP, GRI, MGT, and GS, significant differences were observed in the germination response of seeds for *A. esculentus* and *I. aquatica*. Higher germination rates were observed in seeds of *A. esculentus* and *I. aquatica* treated with

PAW compared to the control (untreated, distilled). This indicated that PAW promoted the germination of seeds. In terms of the germination rate index or seed vigor index, higher GRI was observed in seeds treated with PAW when compared to the control. The germination rate index determined the uniformity and earliness of seed germination. A higher GRI meant that more seeds germinated earlier. Higher GRI was consistently observed in PAW-treated seeds than in untreated seeds, indicating that PAW increased seed vigor.

Seedling characteristics of *A. esculentus* and *I. aquatica* whose seeds were treated with distilled water exposed to APP at varying durations are presented in Table 4. Significant differences were observed in root length, weight, shoot weight, and vigor index I were observed in *A. esculentus* and *I. aquatica*, except for shoot length, while in wheat, all parameters showed significant differences across treatments. In *A. esculentus* and *I. aquatica*, roots were longer in seeds treated with distilled water exposed to APP for 1 minute. Consistently, the shortest root length was observed in the control. Further, in terms of root weight, the heaviest roots were observed in treated seeds. Moreover, in terms of shoot length, similar shoot lengths were observed in *A. esculentus* and *I. aquatica* across treatments. Furthermore, in terms of shoot weight, seedlings derived from PAW-treated seeds had heavier shoots than the control. Lastly, in terms of vigor index I, seeds treated with distilled water exposed to APP for 1 minute consistently had the highest seed vigor value across two species.

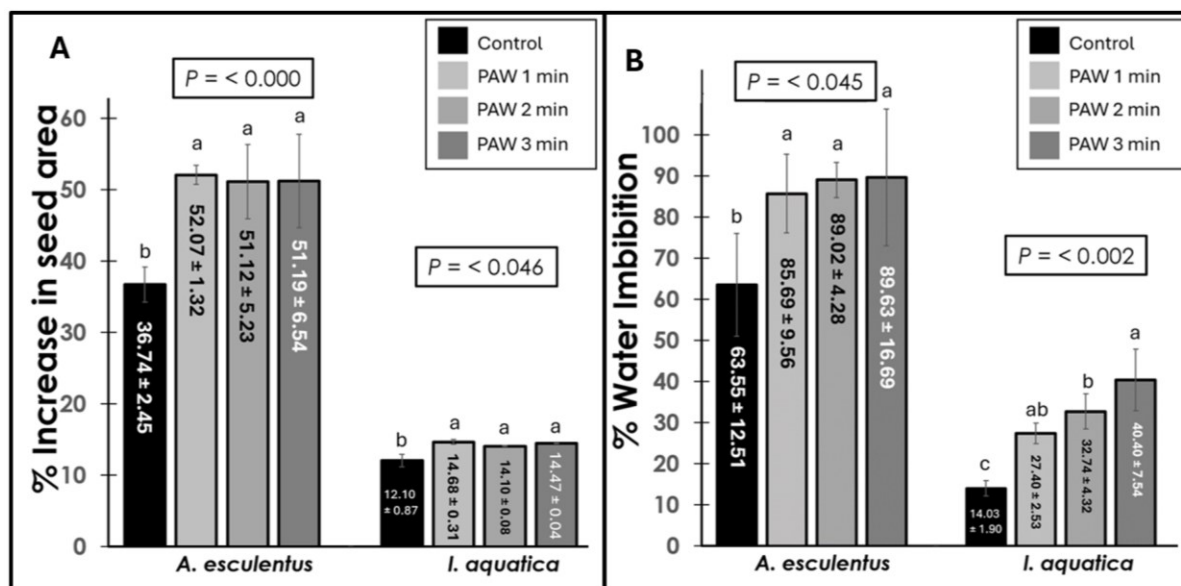


Figure 4. Percentage increase in seed area and percentage of water imbibition. Different letters indicate significant differences in mean values at $P < 0.05$.

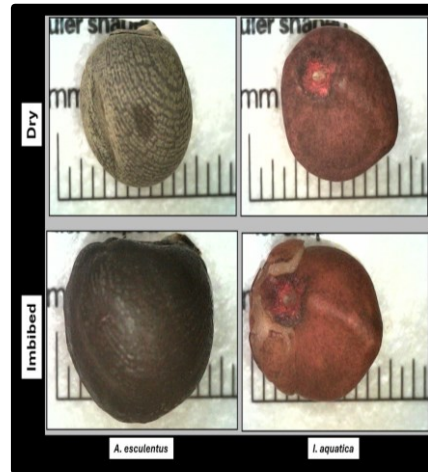


Figure 5. Representative micrographs (40X) of *Abelmoschus esculentus* and *Ipomoea aquatica* seeds before (Dry) and after imbibition with plasma-activated water (Imbibed).

Table 3. Germination characteristics (germination percentage (GP), germination rate index (GRI), mean germination time (MGT), mean germination rate (MGR) and germination speed (GS) of *A. esculentus* and *I. aquatica* seeds across plasma-activated water treatments. Different letters in superscript indicate significant differences in mean values at $P < 0.05$.

Crop Species	Treatments	Germination Characteristics			
		GP (%)	GRI	MGT	GS
<i>Abelmoschus esculentus</i>	Control	54.29 ± 2.86 ^b	6.01 ± 0.49 ^b	3.55 ± 0.35 ^a	4.03 ± 0.57 ^b
	PAW (1 min)	93.33 ± 1.65 ^a	23.63 ± 1.29 ^a	1.98 ± 0.23 ^b	7.63 ± 0.79 ^a
	PAW (3 min)	94.29 ± 2.85 ^a	24.46 ± 1.05 ^a	1.81 ± 0.18 ^b	7.75 ± 0.31 ^a
	PAW (5 min)	88.56 ± 2.86 ^a	23.63 ± 0.64 ^a	1.77 ± 0.15 ^b	7.57 ± 0.54 ^a
	<i>P</i> value	0.000	0.000	0.000	0.000
<i>Ipomoea aquatica</i>	Control	92.38 ± 5.95 ^b	9.49 ± 1.05 ^b	3.71 ± 0.19 ^b	5.88 ± 0.92 ^b
	PAW (1 min)	96.19 ± 3.30 ^a	18.39 ± 0.67 ^a	2.23 ± 0.10 ^a	10.76 ± 0.01 ^a
	PAW (3 min)	96.19 ± 1.65 ^a	21.86 ± 1.39 ^a	1.97 ± 0.22 ^a	10.91 ± 0.47 ^a
	PAW (5 min)	99.05 ± 1.65 ^a	19.96 ± 1.08 ^a	2.30 ± 0.04 ^a	10.39 ± 0.30 ^a
	<i>P</i> value	0.023	0.000	0.010	0.000

Table 4. Root (Rt) and shoot (St), length (L) and weight (Wt), and seedling vigor index (VII) of *Abelmoschus esculentus* and *Ipomoea aquatica* seedlings across plasma-activated water treatments. Different superscript letters indicate significant differences in mean values at $P < 0.05$.

Crop	Treatments	RtL (cm)	RtWt (g)	StL (cm)	StWt (g)	VII
<i>Abelmoschus esculentus</i>	Control	6.27 ± 0.57 ^c	0.01 ± 0.000 ^c	11.50 ± 0.10	0.28 ± 0.06 ^c	963.90 ± 41.17 ^c
	PAW (1 min)	10.0 ± 0.86 ^a	0.04 ± 0.001 ^a	12.97 ± 0.50	0.43 ± 0.05 ^{ab}	2223.33 ± 2.14 ^a
	PAW (3 min)	9.83 ± 0.35 ^{ab}	0.077 ± 0.006 ^a	12.93 ± 1.50	0.57 ± 0.071 ^a	2125.05 ± 19.78 ^{ab}
	PAW (5 min)	8.67 ± 0.55 ^c	0.077 ± 0.015 ^c	13.23 ± 0.31	0.36 ± 0.053 ^{bc}	1941.33 ± 137.86 ^b
	<i>P</i> value	0.000	0.000	0.108	0.007	0.000
<i>Ipomoea aquatica</i>	Control	7.633 ± 0.45 ^c	0.015 ± 0.00 ^c	11.33 ± 0.72	0.14 ± 0.02 ^c	1963 ± 191.65 ^c
	PAW (1 min)	13.27 ± 0.68 ^a	0.043 ± 0.00 ^a	12.3 ± 1.01	0.37 ± 0.05 ^a	2263 ± 82.99 ^a
	PAW (3 min)	9.83 ± 0.35 ^{ab}	0.044 ± 0.01 ^a	10.87 ± 0.81	0.33 ± 0.04 ^{ab}	2088 ± 137.56 ^{ab}
	PAW (5 min)	8.1 ± 0.2 ^b	0.037 ± 0.02 ^b	11.3 ± 0.27	0.29 ± 0.03 ^b	1921 ± 63.24 ^b
	<i>P</i> value	0.001	0.000	0.101	0.001	0.000

DISCUSSION

Characteristics of PAW and Correlation of Different Physical Parameters and Exposure Time to APP

The results of this correlational study corroborate the findings of Sivachandiran and Khacef (2017), who observed a drop in the pH of distilled water from 7.0 to 3.65 during the first minutes of exposure to APP. They also observed an increase in the EC of distilled water after 15 minutes of exposure. This serves as clear evidence of the accumulation of active ions in PAW. A significant increase in the number of active substances like Reactive Nitrogen Species (RNS) and Reactive Oxygen Species (ROS) was observed in plasma-activated water due to the dissolution of ionizing air in water. Various studies have reported similar findings (Benstaali et al. 1998; Oehmigen et al. 2010; Lukes et al. 2012). Sivachandiran and Khacef (2017) further explained that in gas-water environments, like the treatment set-up in this study, the increase in acidity can be attributed to the formation of HNO_2 and HNO_3 acids. In terms of total dissolved solids, Chou and Ting (2023) and Rathore (2022) indicated that ROS and RNS dissolve when they come in contact with distilled water. These dissolved reactive species increase both TDS and EC in distilled water exposed to APP. Chou and Ting (2023) also emphasized that the increase in TDS of distilled water exposed to APP indicates successful plasma activation of the water. Through plasma activation, some compounds were generated, as evidenced by the increasing TDS of PAW, such as hydrogen peroxide (H_2O_2), ozone (O_3), nitrite (NO_2^-), and nitrates (NO_3^-). Furthermore, hydroxyl radicals ($\text{OH}^\cdot$), peroxyxynitrite ($\text{ONOO}^\cdot$), and nitric oxide (NO) are among the typically produced substances, as reported by Wong et al. (2023). Rathore (2022) also observed an increase in dissolved species with increasing exposure time of distilled water to APP.

Resistivity on the other hand, is the ability of a material to resist electric conductivity. Figure 2D shows that R decreases with prolonged exposure to APP. Since there is an increased accumulation of electrically conducting substances in PAW, such as ROS and RONS, as evidenced by the increasing EC and TDS (Figure 2B and 2C), R decreases. The accumulation of ions in the treated water makes it less resistant to EC, hence lowering R.

Effect of PAW on Germination and Seedling Characteristics

The high concentration of RONS in treated distilled water might have caused a higher imbibition rate due to the weakening of the testa, which allowed

greater water absorption. Acid treatment in seeds is commonly used to break dormancy by softening the seed coats, which PAW might have done to the seeds of *A. esculentus* and *I. aquatica*. This likely resulted in more rapid and higher imbibition rates compared to the control. Kučerová et al. (2019) reported that in wheat, increased water uptake was observed when wheat seeds were presoaked in PAW. Another study noted that PAW containing ROS improves seed germination through testa softening due to its acidic nature, while another study found that PAW treatment increased testa pore size, thereby enhancing water uptake (Priatama et al. 2022; Tonmitr and Yonesu 2023).

Guragain et al. (2023) explained that RONS in PAW, such as NO_2 and H_2O_2 may interact with seeds that generally have hydrophobic testa. These RONS interact with the waxy surface of the testa, effectively stripping away the wax. This interaction promotes seed hydrophilicity, initiating quicker water penetration and absorption during the imbibition stage that speed up germination. They also reported that seeds of *Hordeum vulgare* L., *Fagopyrum esculentum* Moench., and *Brassica nigra* (L.) W.D.J. Koch had higher imbibition rates than those soaked in untreated distilled water (Guragain et al. 2021).

The differences in germination characteristics of seeds presoaked in PAW and those soaked only in distilled water can be attributed to the physicochemical parameters of PAW. Studies have shown that the presence of nitric oxide in PAW may have enhanced germination in different plant species such as *Solanum lycopersicum* L., *Capsicum annum* L., *Glycine max* (L.) Merr., and *H. vulgare* (Sivachandiran and Khacef 2017; Guragain et al. 2021; Khan et al. 2023). Pandey et al. (2023) further noted that PAW contains nitrogen compounds important to agricultural production.

Moreover, the presence of various nitrogen species, particularly NO_3^- , stimulates germination by breaking seed dormancy. The RONS in PAW may provide a positive signal that eases dormancy and influences the abscisic/gibberellic acids pathway, which is mainly responsible for germination (Guragain et al. 2021). Khan et al. (2023) elaborated that when NO_3^- is reduced to NO_2^- through nitrate reductase, amino acids may be produced that are vital in plant development, especially during germination. Furthermore, ROS absorbed by the seed during imbibition triggered increased respiration, which initiated sugar oxidation. However, the effect of different PAW exposed to APP at varying ET was found to be insignificant in the germination percentage of *A. esculentus* and *I. aquatica*. Similar responses were observed on seeds of *H. vulgare* and *B. nigra*.

treated with PAW for 5 and 10 minutes (Guragain et al. 2021).

Sivachandiran and Khacef (2017) reported that PAW-treated seeds had shorter shoots than untreated ones. Fan et al. (2020) also observed shorter shoot lengths in PAW-treated mungbean seedlings. Moreover, Sukhani et al. (2021) obtained a similar result in their study on algal growth. They observed higher biomass yield in *Chlorella vulgaris* Beijerinck grown in PAW (Sukhani et al. 2021). In dicots, it is not recommended to have seedlings with leggy shoots, or excessively long shoots, because such shoots are weak allocate energy to elongation rather than in biomass accumulation, making them prone to bending. The increase in biomass accumulation in seedlings is more desirable, which was observed in seedlings derived from PAW-treated seeds.

Kučerová et al. (2019) supported the findings of this study as they observed stimulated growth in wheat seedlings watered with PAW. Moreover, Priatama et al. (2022) reported that in *Arabidopsis*, non-thermal plasma treatment improved germination and long-term growth, as evidenced by increased seedling biomass, enhanced production of antioxidants and phytohormones, and promoted expression of drought tolerance genes. They also stated that the high concentrations of nitrate and nitrite contributed significantly to the improvement of plants irrigated with PAW compared to those plants irrigated with untreated water. Since nitrogen is essential for plant growth and metabolism, APP's ability to increase nitrate and nitrite concentration in water suggests that PAW can act as a nitrogen fertilizer. Given that nitrogen is the backbone of metabolism, plant organ development in the test species improved under PAW treatment compared to the control. Despite not having variable responses across treatments on shoot length, shoots were heavier on PAW-treated plants. Song et al. (2023) reported that PAW-treated peanut seeds had improved biomass-related morphological traits, particularly hypocotyl growth. At the cellular level, they observed enlarged parenchyma cells in the hypocotyl pith of PAW-treated seeds. Further, qRT-PCR showed upregulation of auxin response and growth-related genes such as AhGI 75, AhCUC3-65, and AhARF5. The upregulation of these genes promotes cell division, thereby increasing biomass.

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GENERATIVE AI STATEMENT

The authors did not use generative AI tools while writing the paper.

ETHICAL CONSIDERATIONS

The study was exempted from any ethics guidelines. Human respondents and experimental animals were not involved in the study. However, safety protocols in handling plasma were strictly observed.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no competing interests among the authors.

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