

Improving Germination of Mustard (*Brassica nigra*) and Bok choi (*Brassica rapa* subsp. *chinensis*) through Priming using Plasma Activated Water (PAW)

Alangelico San Pascual^{1*}, Catherine Joy Dela Cruz^{1,3}, Maurice Gravidez², and Ana Maria Chupungco¹

¹Research Unit, Department of Science and Technology (DOST) - Philippine Science High School Main Campus, Quezon City, Metro Manila, Philippines; ²Science Unit, De La Salle Araneta University - Integrated School, Malabon City, Metro Manila, Philippines; ³Energy Engineering Program, College of Engineering, University of the Philippines Diliman, Quezon City, Philippines. *Corresponding author, aosanpascual@pshs.edu.ph

The study explores Plasma Activated Water (PAW) or water directly exposed to Atmospheric Pressure Plasma (APP) as priming agent to improve seed germination and vigor of Mustard and Bok Choi. Seeds were primed using distilled water exposed to APP for two minutes (Treatment 1) and five minutes (Treatment 2) using APP jet. These treatments were compared to positive control (untreated distilled water, Treatment 3) and negative controls (no priming, Treatment 4). The parameters on the priming agent (PAW) observed were physicochemical properties (pH, electric conductivity (EC), total dissolved solids (TDS), resistivity, and oxidation reduction potential (ORP)). Seeds were soaked in the priming agent for 6hr followed by 8hr air-drying before seed germination test using top-of-paper (TP) method. The study was laid out using CRD, replicated thrice with two (2) subsamples with 100 seeds per subsample. Germinated seeds were counted from first to seventh day. Seed germination, germination rate index, mean germination time, and germination speed were measured. Data was analyzed using ANOVA and Tukey's Honest Significant Difference. In terms of PAW's properties, both PAW are acidic, with high EC, TDS, ORP and low resistivity as compared to untreated distilled water. Significantly, higher germination percentage, more uniform and faster germination with shorter germination time were observed on seeds presoaked in PAW (regardless of water exposure to APP) compared to the controls for both Mustard and Bok Choi. This shows that PAW is effective as priming agent to increase germinability and vigor of Bok Choi and Mustard. Specifically, distilled water exposed to APP for 2 mins is sufficient to improve germination and vigor of Mustard and Bok choi seeds.

Keywords: Atmospheric Pressure Plasma (APP), *Brassica napus*, chinese cabbage, Hydro-Priming Plasma Activated Water (paw), plasma agriculture

INTRODUCTION

Seed quality evidenced by high and uniform germination rate and high seedling quality are vital for the harvest and production success and attainment of food security (Chand et al. 2018). Pre-planting activities such as seed germination in combination with the genetic capacity of the variety are critical in efficient and sustainable food production (Fu et al. 2024). These pre-germination activities, most especially seed treatments and germination are often faced with biotic and abiotic challenges such as suboptimal storage, low seed viability, seedborne pathogens, and poor seed vigor (Jose et al. 2023). Given these problems, several ways to enhance germination and seedling success were done including seed invigoration techniques specifically seed priming (Devika et al. 2021). Seed priming is the process of controlled hydration of seeds to a level that permits pre-germinative metabolic activity to proceed but prevents the actual emergence of the radicle (Raj et al. 2019). It is commonly used by seed producers and growers to regulate germination. As a result, fast and uniform emergence can be expected among the primed seeds due to the early completion of germination events (Zulfiqar 2021). Seed priming techniques target pre-germinative metabolic activities rooted in seed germination, seedling vigor, and plant

performance during the vegetative stage (Sukhani et al. 2021). There are multiple priming agents already published and tested to improve certain parameters, such as growth, vigor, defense mechanism and tolerance to pathogens including essential oils (Cejalvo and Mercado 2018), organic fertilizer (Kaya and Coskun 2020), bacterial metabolites (Rivarez et al. 2021), rice wash (Gravidez et al. 2024), among others. Other studies have included the use of ionizing radiation (Wong et al. 2023), ultrasonic waves (Lopez-Ribera and Vicient, 2017), frequency-specific sound signals (Vicient 2017), cold plasma (Rasooli et al. 2021), atmospheric pressure plasma (Sivachandiran and Khacef 2017), and materials exposed to plasma from different sources such as atmospheric pressure plasma and one example of these materials is plasma activated water (PAW) (Dela Cruz et al. 2024).

Atmospheric Pressure Plasma (APP) as defined by Zhou et al. (2019) is "a partially ionized gas, consisting of charged particles, reactive species, electric fields, and ultraviolet photons". These reactive species, either Oxygen (ROS) or Nitrogen (RONS) Reactive species generated by APP could react with water to produce plasma activated water (PAW) which has promising effect and uses. Some used PAW for sterilization

because of its outstanding antibacterial ability. Shen et al. (2016) that PAW has antibacterial effect on important microorganisms like *Escherichia coli*, *Saccharomyces cerevisiae*, *Hafnia alvei*, *Staphylococcus aureus*, and *Candida albicans*.

PAW can be produced by exposing water to cold plasma discharge from an atmospheric plasma jet. This results in water with increased amounts of reactive oxygen and nitrogen species, lower pH, and higher conductivity which may affect pathogen growth and plant tolerance to the disease, and metabolic activities, like germination, growth, development, ripening and delaying of senescence (Usman et al. 2023). It emerged as a priming agent to enhancing seed germination and seedling vigor. Several studies have been done to test the effectiveness of PAW using different APP discharge through different configurations like corona discharges, dielectric barrier discharges (DBD), and plasma jet (APPJ). Studies using PAW as priming agent were done on mungbean using APPJ (Fan et al. 2020), soybean and wheat using gliding arc discharge (Guragain et al. 2021), and in Rice using DBD (Bian et al. 2024) and show that PAW was able to improve the germination and germination vigor of the important crops. However, no studies were done in the Philippines in using APP through a plasma jet (APPJ) to produce PAW and treat important crops like vegetables. This may help farmers to increase the viability of seeds which are commonly stored in suboptimal conditions. These seeds sub-optimally stored show low viability, and this study may provide understanding on how PAW be utilized in seed priming by characterizing their physical properties and utilize them in germination tests. By elucidating the effects of PAW on germination of two crucifers, this study contributes to develop newer agricultural practices to enhance crop productivity, minimize seed losses, and promote adoption of eco-friendly technologies.

Chinese cabbage also known as bok choy and mustard (*Mustasa*) are economically important crucifer species in the Philippines. These are commonly grown in Asia; its petioles and green leaves are utilized as important ingredients to various Asian dishes. In the Philippines, bok choy and other brassicas like mustard provide additional income to low land rice farmers as component of the crop rotation or multiple cropping scheme after rice harvesting. Farmers also plant bok choy and mustard as cash crops given their short growing period that they can plant in between rice planting which may also be done to efficiently utilizing excess water and fertilizer left by rice production. Considering the high demand for bok choy and mustard combined with its importance in Filipino low land rice cropping systems, application of priming techniques for efficient, fast, high-quality, and uniform germination must be done to ascertain production success.

The study aims to determine the effectiveness of plasma activated water (PAW) as a priming agent for mustard and bok choy. Specifically, the study aims to a) determine the physical characteristics of distilled water exposed to atmospheric pressure plasma (APP) for 2 and 5 min and compare it with untreated distilled water. Further, it aims to b) determine the germination response of mustard and bok choy seeds treated with

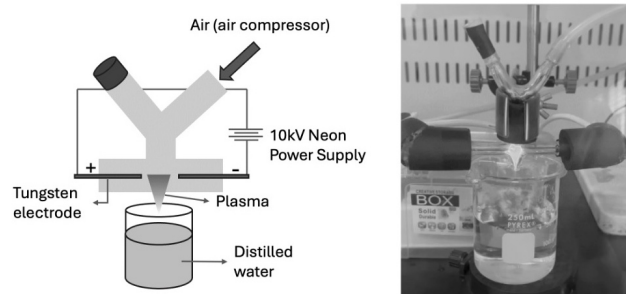


Figure 1. (a) Schematic of the Atmospheric Pressure Plasma Jet (APPJ) Set-up (Park et al. 2013 and Malapit and Baculi, 2021). (b) Actual distilled water treatment. Distilled water (200 mL) was exposed to APP with a nozzle to water distance at approximately 5 cm at 1, and 5 min for the seed application (Dela Cruz et al. 2024).

distilled water exposed to APP for 2 and 5 min compared to seeds soaked in untreated distilled water and unprimed seeds.

MATERIALS AND METHODS

Preparation of distilled plasma-activated water

An Atmospheric pressure plasma jet (Park et al. 2013; Dela Cruz et al. 2024, and Malapit and Baculi, 2021) was used to deliver the plasma to the distilled water (Figure 1). The jet has two 0.5 mm diameter tungsten (W) rods as electrodes. These are enclosed in a 14 mm outside diameter glass tube with rubber stoppers. The spacing between the W rods is approximately 10 mm which was connected to a 10 kV 30 mA neon light power supply. The upper part of the custom-built glass tube has two openings as gas inlets. One was covered with a rubber stopper while the other is connected to an air compressor that outputs 55 L min⁻¹ (Dela Cruz 2024).

Characterization of distilled plasma-activated water

For the measurements of physical properties of distilled plasma-activated water at 2- and 5-min exposure times, three trials were prepared, and the measurements included pH, electrical conductivity (EC), resistance, total dissolved solids (TDS), and Oxidation Reduction Potential (ORP). A handheld automatic water quality meter (YY-1010) was used to measure pH, TDS, EC ($\mu\text{S cm}^{-1}$), and resistivity (M Ω). The resolution and accuracy of pH, EC, TDS, temperature, and resistivity are 0.01 pH $\pm$ 0.02 pH, 1 $\mu\text{S cm}^{-1}$ $\pm$ 2% full-scale temperature, 1 ppm $\pm$ 2% full-scale temperature, and 0.1 M Ω $\pm$ 1% full scale temperature, respectively.

Preparation of seed treatments

The experiments were laid out in a completely randomized design (CRD) with three replicates and two subsamples each replicate for the germination test. Two subsamples of 100 seeds were used for three replicates for four treatments for a total of 2400 seeds per species (2 subsamples x 100 seeds x 3 replicates x 4 treatments). There were three pre-soaking treatments (APP-treated distilled water with 2- and 5-min exposure duration) and positive control

(untreated distilled water) and negative control (no priming). Seeds of mustard and bok choy used in this study were sourced from the Bureau of Plant Industry in Quezon City, Philippines.

Presoaking method and germination test

Seeds without physical damage were used in the study. Seeds were presoaked in different treatments for 8 hr at room temperature. Afterwards, the seeds were blot-dried using tissue paper. Seeds were further air-dried for 8 hr before being subjected to germination tests using the top-of-paper method. A day after sowing, manual counting of germinated seeds was done. A germinated seed is characterized by having its radicle protrude from the seeds (> 2mm). Observation was done until the 7th day of germination. Germination percentage (GP%), germination rate index (GRI), mean germination time (MGT), and germination speed (GS) were computed.

Percent germination (%) (GP). Two subsamples with one hundred seeds per subsample for three replicates were sown in moistened paper towels and equidistantly placed on top of moist tissue paper. The set-up was sprayed with enough distilled water during the testing period of 7 days to keep the set-up moist. Counting of seedlings was done daily at 5:00 PM. The higher germination percentage means higher seed viability. GP was calculated using the formula shown in Table 1.

Germination Rate Index (GRI). This is a parameter in combination with both the germination percentage and germination time. Daily counting of germinated seeds was done from day 1 to day 7. GRI was computed using the formula in Table 1. Further, the higher germination rate index indicates higher seed vigor which specifically means earlier yet uniform germination.

Mean Germination Time (MGT). Mean germination time is a measure of the time it takes for the seed to germinate, focusing on the day at which most seeds have germinated. The lower MGT, the shorter is the time for seeds to germinate. MGT was calculated using the formula in Table 1.

Germination Speed (GS). This is the rate of germination in terms of the total germinated seeds in each time frame. Further, the higher the germination speed, the faster the rate of germination. The data from daily germination was used to calculate GS using the formula found in Table 1.

Statistical Analysis

The data on the physical parameters of PAW were collected in 5 replications with 3 subsamples per replicate. The data on the physical parameters of PAW and Seed germination data were analyzed using the Analysis of Variance (ANOVA) after meeting assumptions of normality and homoscedasticity. Afterwards, pairwise mean comparison was done using the Least Significant Difference (LSD) test at a 5% level of significance. Analysis was done using Jamovi® software.

Table 1. Summary of the formulas used in the study

Parameter/unit	Formula
Germination Percentage (%)	$((\text{Total no. of seedlings}) / (\text{No. of seeds sown})) \times 100$
Germination Rate Index	$n/1 + \dots + n/7$; $n = \text{no. of seedlings}$
Mean Germination Time (days)	$\sum (\text{Daily no. of germinated seeds} \times \text{no. days from beginning of test}) / (\text{Total germinated seed at final count})$
Germination Speed (seedling/day)	$(\text{Total no. of seedlings at Day}_3 / \text{Day}_3) + (\text{Total no. of seedlings at Day}_7 / \text{Day}_7)$

RESULTS AND DISCUSSION

Determination of physicochemical characteristics of distilled water exposed and unexposed to Atmospheric Pressure Plasma.

The physicochemical characteristics of distilled water unexposed to APP and distilled water exposed to APP for 2 and 5 min were observed (Table 2). Significant differences were observed in all physical parameters measured on untreated and APP- treated distilled water. In comparison with untreated distilled water, both batches of distilled water exposed to APP for 2 and 5 min were more acidic. The control (distilled water unexposed to APP) has a pH of 6.64 while distilled water exposed to APP for 2 and 5 min has a pH of 3.24 and 3.0, respectively. In terms of oxidation reduction potential, increased oxidative potential was observed on APP- treated distilled water when compared with the control. Distilled water exposed to APP for 2 and 5 min have ORP of 462 and 662 V, respectively while the untreated has an ORP of 196 V. However, in terms of resistivity, highest resistivity was observed in the control (4813 MΩ) and low resistivity was observed in both distilled water batches exposed to APP for 2 (53.5 MΩ) and 5 min (30.20 MΩ). EC increased from 0.667 S/m for the untreated distilled water to an average of 253 and 472 S/min for distilled water exposed to APP for 2 and 5 min, respectively. While TDS increased from 0 to 125 and 234 ppm for untreated distilled water and distilled water exposed to APP for 2 and 5 min, respectively.

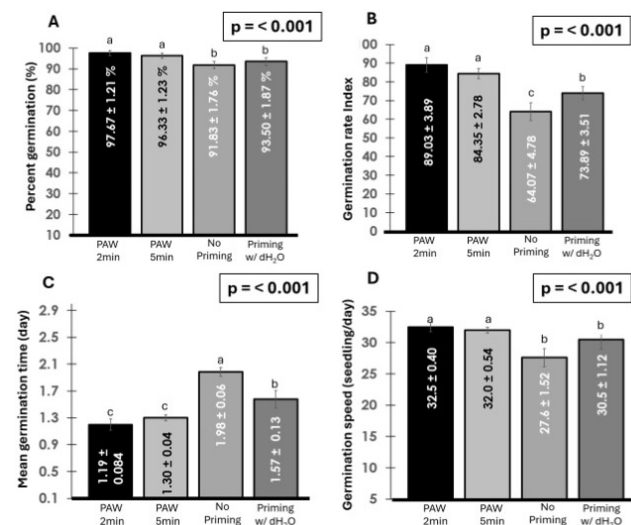
Changes in physicochemical parameters of plasma activated water were reported by multiple authors like Abbaszadeh et al. (2021), Sivachandiran and Khacef (2017), Rathore et al. (2022), and Wong (2023), among others. Abbaszadeh et al. (2021) explained that when water is exposed to APP, plasma discharges generate reactive oxygen and nitrogen species hence the water's acidic pH. In this present study, even short exposure of 2 min of water to APP, there was a high change of pH from alkaline pH of 6.64 to acidic pH of 3.24.

The study of Rathore et al. (2022) stated that the increase in EC of PAW, decrease in pH value and increasing acidity are due to the formation of hydrogen peroxide species, nitric and peroxyntous acid (Thirumdas et al. 2018). The high presence of ions and reactive species in PAW upon plasma activation of

Table 2. Physicochemical characteristics of distilled water exposed and unexposed to APP.

Physical parameters	Duration of exposure to APP		
	0	2 minutes	5 minutes
pH	6.64 ± 0.49 ^a	3.24 ± 0.10 ^b	3.00 ± 0.23 ^b
Oxidative Reduction Potential (V)	196.33 ± 1.52 ^c	462 ± 20.82 ^b	662 ± 11.36 ^a
Resistivity (MΩ)	4813 ± 0.65 ^a	53.50 ± 1.82 ^b	30.20 ± 0.58 ^c
Electric conductivity (S/m)	0.667 ± 1.15 ^c	253 ± 1.15 ^b	472 ± 12 ^a
Total Dissolved Solids (ppm)	0 ^c	125.67 ± 4.93 ^b	234 ± 6.0 ^a

*Means with different letters are significantly different at α=5%.

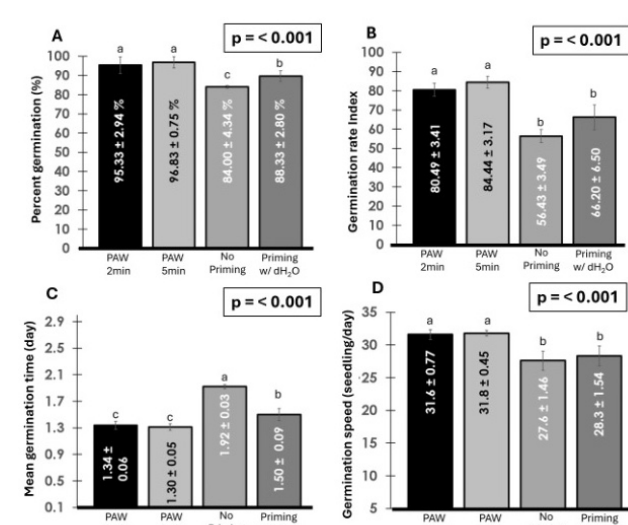


*Bars with different letters are significantly different at α=5%;

Figure 2. Average Percent germination (A), germination rate index (B), mean germination time (C) and germination speed (D) of mustard seeds primed using distilled water exposed to APP in 2 and 5 min in comparison to positive (primed using untreated distilled water) and negative (unprimed seeds) controls.

water is also the cause of the increase of electric conductivity of PAW (Silapasert et al. 2023). Furthermore, Rathore et al. (2022) explained that since there is an increase in nitrate and nitrite ions in water as it was plasma activated, there is also an increase in the production of electric conducting species which was evident on the increased TDS on PAW compared to untreated distilled water. Moreover, the presence of oxidizing species also increased the ORP of PAW. Rathore et al (2022) stated that in PAW, Hydrogen peroxide (H₂O₂), dissolved Ozone (O₃), hydroxide (OH⁻) ions, peroxyxynitrite (ONOO⁻), nitrate and nitrite ions increase oxidation and reduction potential (ORP) of Plasma activated water. On the other hand, the low resistivity, or the ability of the material, fluid etc. to resist electric current, can be attributed to the increasing concentration of reactive oxygen and nitrogen species and other electric conducting species present (Dela Cruz et al. 2024).

In addition to the significant changes observed in the physicochemical parameters of distilled water exposed to APP through APPJ (Park et al. 2013), this study also tested the effect of PAW on seed germination. Furthermore, two economically important cruciferous



*Bars with different letters are significantly different at α=5%;

Figure 3. Average Percent germination (A), germination rate index (B), mean germination time (C) and Germination speed (D) of bok choi seeds primed using distilled water exposed to APP in 2 and 5 min in comparison to positive (primed using untreated distilled water) and negative (unprimed seeds) controls.

crops in the Philippines, bok choi and mustard, were used in the experiments.

Determination of germination parameters of mustard and bok choi seeds primed with and without PAW.

Germination percentage (%GP) and germination rate index (GRI), mean germination time (MGT), and germination speed (GS) of mustard seeds primed using distilled water exposed to Atmospheric pressure plasma (APP) in 2 and 5 min compared with the positive (priming using untreated distilled water) and negative control (no priming) were shown (Figure 2).

Significant differences were observed in the germination percentage (p<0.001) where the highest germination percentages were observed on mustard seeds treated with distilled water exposed to APP for 2 (97.67%) and 5 min (96.33%) (Figure 2A).

In terms of germination rate index (Figure 2B), significant differences were observed (p= < 0.001) across treatments. Mustard seeds treated with PAW (2 min) and PAW (5 min) had highest germination rate index at 89.03% and 84.35%, respectively, followed by

seeds that are primed using untreated distilled water (73.89) and the lowest GRI was observed on seeds that did not receive seed priming (64.07).

Further, significant differences were observed among treatments for both mean germination time (Figure 2C) and germination speed (Figure 2D). Shortest mean germination time was observed on primed seeds with distilled water exposed with APP for 2 min (1.19 days) and 5 min (1.30 days). Longest mean germination time were observed on the seeds primed with untreated distilled water (1.57 days) and seeds that were not primed (1.98 days). However, in terms of germination speed, seed treated with distilled water exposed to APP for 2 (32.5 seedling day⁻¹) and 5 min (32.0 seedling day⁻¹) were different to the negative control or unprimed seeds (27.6 seedling day⁻¹) and to the positive control (30.5 seedling day⁻¹).

Germination percentage and germination rate index of bok choy seeds primed using distilled water exposed to APP in 2 and 5 min compared with the positive (priming using untreated distilled water) and negative control (no priming) (Figure 3A and 3B). Significant differences were observed in the germination percentage where the highest germination percentages were observed on seeds treated with distilled water exposed to APP for 2 (95.33%) and 5 (96.83%) min. In terms of germination rate index, significant differences were observed ($p < 0.001$) across treatments. Bok choy seeds treated with PAW (2 min) and PAW (5 min) had highest GRI at 80.49 and 84.44, respectively. This is followed by seeds that are primed using untreated distilled water (66.20) and on seeds that did not receive seed priming (56.43).

Mean germination time (Figure 3C) and germination speed (Figure 3D) of bok choy seeds primed using distilled water exposed to APP for 2 and 5 min compared with the positive (hydropriming) and negative control (no priming) were shown. Significant differences were observed among treatments for both variables. Shortest mean germination time was observed on primed seeds with distilled water exposed with app for 2 (1.34 days) and 5 min (1.30 days). Longest germination time were observed on the seeds that received no priming treatment (1.92 days) followed by seeds primed using untreated distilled water (1.50 days). However, in terms of germination speed, seed treated with distilled water exposed to APP for 2 (31.6 seedling day⁻¹) and 5 min (31.8 seedling day⁻¹) were different to the negative control or seeds that did not underwent priming at all (27.6 seedling day⁻¹) and seeds that were primed using untreated distilled water (28.3 seedling day⁻¹).

Multiple studies have investigated the effects of Plasma activated water on seed germination. PAW was tested and reported to improve germination in Lettuce, mung bean, soybean, wheat, papaya, and corn among others (Rathore et al. 2023; ISAA Inc. (2024); Wang (2023); and Gebremical et al. (2023). In black gram seeds, germination was improved by 10-15% after treatment with PAW (Sajib et al. 2020).

In this present study, it was observed that PAW, regardless of exposure time to Atmospheric pressure plasma through the plasma jet have increased

germination and vigor of seeds. Increased seed vigor indicated earlier and more uniform germination. Further, seeds presoaked in PAW had shorter germination time and faster germination speed. Therefore, PAW did not only improve seed's germinability, it also hasten, ascertain germination and improved synchrony and uniformity.

Several studies have provided explanations as to how germination was improved by using plasma activated water (PAW). The changes in the physicochemical characteristics of PAW have caused the improvement of germination of both mustard and bok choy when they were presoaked to PAW. Among these changes in physicochemical, acidity of PAW is one important factor to investigate. Low pH allows increase in water permeability of the seed coat hence also increased the seeds' capacity to imbibe water that leads to faster germination (Attri et al. 2020). Vecino-Bueno et al. (2009) reported that there was improvement in germination of *Erica andevalensis*, a plant endemic to Spain, after acid treatment. Additionally, treatment of acid with pH of 2 did not show germination of *E. andevalensis* but there was an improved germination of the seeds when these were treated with a solution with a pH of 3 to 5. The pH of the solution reported by Vecino-Bueno et al. (2009) is similar to the pH of PAW exposed to APP for 2 and 5 min which has a pH of 3.62 and 3.2, respectively.

Adhikari et al. (2020) elaborated that one mechanism of plasma to the seed is through the changes in physical and chemical properties of the seed coat. The changes caused by interaction of water with high acidity and high electric conductivity resulted in increased hydrophilicity and water permeability. This promotes water imbibition which is one of the initial phases of germination. Mujahid et al. (2020) also explained that hydroxyl radicals, which are present in Plasma Activated water promoted cell wall softening. Further, Souza (2001) stated that plasma treatment also increased pore size, increasing water imbibition, and leading to seed genetic regulation and finally, seed germination.

Furthermore, Sehwat et al. (2017) stated that the removal of lipid layer of the seed due to plasma treatment also promoted germination. Perez-Piza et al. (2018) also discussed that the lipid layer in the seed coat undergo oxidation which improves the seeds' affinity to water. More than the effect of acidic nature of PAW to the seed coat integrity, Mueller et al. (2009) as cited by Waskow et al. (2021) also discussed that RONS causes cleavage of cell wall polymers. At the molecular level, Bafoil et al. (2018) also explained that there is an increased expression of peroxidase-related genes after seeds are soaked in PAW. These genes are responsible for regulating hydrogen peroxide, which initiates rupture of testa. Further, increased nitrate concentration and other nitrogen ions, hydrogen peroxide and low water pH were found to also increase seed germination (Sivachandiran and Khacef 2017).

Abbaszadeh et al. (2024) also stated that in lettuce, PAW improved germination significantly. They explained that the nitrate, nitrite, and hydrogen peroxide ions present in PAW have caused activation

of different growth signaling pathways that also provided a nutritional role to PAW as explained by Kucerova et al. (2019). Further, the presence of Hydrogen peroxide and nitrate ions may have terminated seed dormancy that then hastened germination (Judee et al. 2018).

CONCLUSION AND RECOMMENDATION

Water treated with APP had significantly increase its pH, ORP, TDS, and EC. Higher acidity, EC, TDS, and ORP but low resistivity was observed on distilled water exposed to APP for 5 min followed by distilled water exposed to APP for 2 min. However, highest resistivity, low EC, alkaline pH, and low TDS were observed on the untreated distilled water. The increased in different reactive species bought by APP exposure was the reason for the physicochemical changes. However, seeds presoaked with PAW regardless of its exposure time improved germination in terms of its speed, rate, and uniformity. The results show the potential of PAW to improve seed germination of bok choy and mustard. Lastly, it was observed that the minimum APP exposure time of distilled water to affect the germination of bok choy and mustard was found to be 2 min. It is then recommended that PAW may be used as a priming treatment. Future studies may be done with shorter exposure time to APP to further ascertain the minimum exposure time for it to enhance germination. Lastly, other studies may be done to explore seedling characters and even yield of these cruciferous crops.

ACKNOWLEDGEMENT

The authors are most grateful to the Ateneo Vacuum Coating and Plasma Physics Laboratory of Ateneo de Manila University, specifically to Mr. Ivan Culaba for the design of the APPJ and Dr. Christian Lorenz S. Mahinay for the donation of a whole APPJ system to Ms. Catherine Joy M. Dela Cruz of the PSHS-MC Research Unit. Also, the authors are most grateful to Mr. Elijah Estante, Mr. Gerald Tiburcio and Mr. Ian Carlos Robles for the various assistance during the conduct of the experiments and writing of the manuscript.

REFERENCES

- Adhikari B, Adhikari M, Park G. 2020. The effects of plasma on plant growth, development, and sustainability. *Applied Sciences*. 10(17):6045. <https://doi.org/10.3390/app10176045>.
- Abbaszadeh R, Khosravi Nia P, Fattahi M, Marzdashti HG. 2021. The effects of three plasma-activated water generation systems on lettuce seed germination. *Res. Agr. Eng*. 67:131–137.
- Attri P, Ishikawa K, Okumura T, Koga K, Shiratani M. 2020. Plasma agriculture from laboratory to farm: A Review. *Processes*. 8(8):1002. <https://doi.org/10.3390/pr8081002>.
- Bafoil M, Jemmat A, Martinez Y, Merbahi N, Eichwald O, Dunand C, Yousfi M. 2018. Effects of low temperature plasmas and plasma activated waters on *Arabidopsis thaliana* germination and growth. *PloS one*, 13(4). e0195512.
- Bian JY, Guo XY, Lee D, Sun XR, Liu LS, Shao K, Liu K, Sun HN, Kwon T. 2024. Non-thermal plasma enhances rice seed germination, seedling development, and root growth under low-temperature stress. *Appl Biol Chem*. 67(2). <https://doi.org/10.1186/s13765-023-00852-9>.
- Cejalvo R, Mercado M. Presoaking Treatment of soybean [*Glycine max* (L.)] seeds using fermented plant extracts and commercial liquid fertilizer. *JPAIR Multidisciplinary Research*. 34: 40-56.
- Chand H, Bishnu K. 2018. Quality seeds for food security and food self-sufficient during haved of Covid-19 in Nepal. *Tropical Ecosystems*.1(2): 71-78.
- Dela Cruz C, San Pascual A, Chupungco A, Gravidez M. 2024. Enhancing seed germination of chinese cabbage using water treated with atmospheric pressure plasma (APP) in varying exposure times. Presented during the De La Salle University - ARCHERS. May 16-17, 2024.
- Devika OS, Singh S, Sarkar D, Barnwal P, Suman J, Rakshit A. 2021. Seed priming: a potential supplement in integrated resource management under fragile intensive ecosystems. *Front Sustain Food Syst*. 5:654001.
- Fan L, Xiufang L, Yunfang M, Qisen X. 2020. Effects of plasma-activated water treatment on seed germination and growth of mung bean sprouts. *J Taibah Univ for Sci*. 14: 823-830. doi: 10.1080/16583655.2020.1778326.
- Fu Y, Ma L, Li J, Hou D, Zeng B, Zhang L, et al. 2024. Factors influencing seed dormancy and germination and advances in seed priming technology. *Plants*. 13:1319.
- Gebremical G, Tappi S, Laurita R, Capelli, F, Drudi F, et al. 2023. Effects of plasma activated water (PAW) on rheological, thermal, hydration and pasting properties of normal maize, waxy maize and potato starches. *Food Hydrocolloids*. 144. 109006. <https://doi.org/10.1016/j.foodhyd.2023.109006>.
- Gravidez M, Rejuso PJ, Batara F, et al. 2024. Exploring the potential of white rice wash in promoting germination and vigor of eggplant and papaya seeds. 58th BIOTA Annual Meeting and Scientific Convention. April 18-20, 2024. Boracay, Aklan.
- Guragain R, Pradhan S, Baniya H, Pandey B, et al. 2021. Impact of plasma-activated water (PAW) on seed germination of soybean. *Hindawi Journal of Chemistry*. 7517052.

- [ISAA.Inc.] International service for the acquisition of agri-biotech applications. 2024. Plasma-Activated water boosts germination and growth of mungbeans. <https://www.isaaa.org/kc/cropbiotechupdate/article/default.asp?ID=20585>. Date accessed: September 6, 2024.
- Jose J, Mercado M, de Guzman L. 2023. Germinability and vigor of partially-aged corn (*Zea mays* L.) seeds presoaked in Sampaguita (*Jasminum sambac* [L.] Aiton) flower extract and essential oil. *International Journal of Agricultural Technology*. 19: 1553-1556.
- Judée F, Simon S, Bailly C, Dufour T. 2018. Plasma-activation of tap water using DBD for agronomy applications: Identification and quantification of long lifetime chemical species and production/consumption mechanisms. *Water research*. 133: 47–59.
- Kaya A, Coşkun N. 2020. Effect of organic fertilizer forms & doses on seed germination & seedling development of rapeseed. *Applied Ecology and Environmental Research*. 18(5):6813-6828.
- Kučerová K, Henselová M, Slováková L, Hensel K. 2018. Effects of plasma activated water on wheat: Germination, growth parameters, photosynthetic pigments, soluble protein content, and antioxidant enzymes activity. *Plasma Processes and Polymers*. e1800131.
- López-Ribera I, Vicient CM. 2017. Use of ultrasonication to increase germination rates of Arabidopsis seeds. *Plant Methods*. 13: 31.
- Mai-Prochnow A, Zhou R, Zhang T, et al. Interactions of plasma-activated water with biofilms: inactivation, dispersal effects and mechanisms of action. *npj Biofilms Microbiomes* 7, 11.
- Malapit GM, Baculi RQ. 2021. Bactericidal efficiency of silver nanoparticles deposited on polyester fabric using atmospheric pressure plasma jet system. *The Journal of The Textile Institute*. 113(9): 1878–1886. <https://doi.org/10.1080/00405000.2021.1954426>
- Müller K, Hess B, Leubner-Metzger G. 2007. A role for reactive oxygen species in endosperm weakening. in: adkins sw, navie sc, ashmore s, editors. *germination and dormancy of seeds: Biology, Development and Ecology*. Wallingford: CAB International. p. 287.
- Mujahid Z, Tounekti T, Khemira H. 2020. Cold plasma treatment to release dormancy and improve growth in grape buds: a promising alternative to natural chilling and rest breaking chemicals. *Sci Rep*. 10, 2667. <https://doi.org/10.1038/s41598-020-59097-x>.
- Pérez-Pizá M, Prevosto L, Grijalba P, Zilli C, Cejas E, Mancinelli B, Balestrasse K. 2019. Improvement of growth and yield of soybean plants through the application of non-thermal plasmas to seeds with different health status. *Heliyon*. 5(4). e01495.
- Raj A, Sheeja R. 2019. Seed priming: An approach towards agricultural sustainability. *Journal of Applied and Agricultural Science*. 11: 227-234.
- Rasooli Z, Barzin G, Mahabadi T, Entezari M. 2021. Stimulating effects of cold plasma seed priming on germination and seedling growth of cumin plant. *South African Journal of Botany*. 142:106-113
- Rathore V, Tiwari B, Nema S. 2022. Treatment of pea seeds with plasma activated water to enhance germination, plant growth, and plant composition. *Plasma Chem Plasma Process*. 42:109–129.
- Rivarez M, Parac E, Dimasingkil S. et al. 2021. Influence of native endophytic bacteria on the growth and bacterial crown rot tolerance of papaya (*Carica papaya*). *Eur J Plant Pathol*. 161, 593–606.
- Sivachandiran L, Khacef A. 2017. Enhanced seed germination and plant growth by atmospheric pressure cold air plasma: combined effect of seed and water treatment. *RSC Adv*. 7:1822-1832.
- SJ Park, GMQ Saquilayan, MA Jallorina, IB Culaba, Optical analysis of argon atmospheric plasma jet, *Proceedings of the Samahang Pisika ng Pilipinas* 31, SPP2013-5A-7 (2013). URL: <https://proceedings.spp-online.org/article/view/SPP2013-5A-7>.
- Sajib S, Billah M, Mahmud S. et al. 2020. Plasma activated water: the next generation eco-friendly stimulant for enhancing plant seed germination, vigor and increased enzyme activity, a study on black gram (*Vigna mungo* L.). *Plasma Chem Plasma Process* 40, 119–143.
- Shen J, Tian Y, Li Y Ma R, Zhang Q, Zhang J, Fang J. 2016. Bactericidal effects against *S. aureus* and Physicochemical properties of Plasma Activated Water stored at different temperatures. *Sci Rep*. 6: 28505. doi: 10.1038/srep28505
- Sehrawat R, Thakur A, Vikram A, Vaid A, Rane R. 2017. Effect of cold plasma treatment on physiological quality of okra seed. *Journal of Hill Agriculture*. 8. 66.
- Silapasert P, Yatongchai C, Sarapirom S. 2023. Investigation of plasma activated water in the growth of green microalgae (*Chlorella* spp.). *Journal of Physics: Conference Series*. 2431.

- Sukhani S, Punith N, Ekatpure A, Salunke G, Manjari M, et al. 2021. Plasma-activated water as nitrogen source for algal growth: A microcosm study, in IEEE Transactions on Plasma Science. 49: 551-556.
- Than H, Pham T, Nguyen D, et al. Non-thermal plasma activated water for increasing germination and plant growth of *Lactuca sativa* L. Plasma Chem Plasma Process 42, 73–89.
- Thirumdas R, Kothakota A, Annapure U, Siliveru K, Blundell R, Gatt R, Valdramidis V. 2018. Plasma activated water (PAW): Chemistry, physico-chemical properties, applications in food and agriculture. Trends in Food Science & Technology. 77:21-31.
- Usman I, Afzaal M, Imran A, Saeed F, Afzal A, et al. 2023. Recent updates and perspectives of plasma in food processing: a review. International Journal of Food Properties. 26:552-566.
- Vecino-Bueno I, Redondo-Gomez S, Figueroa M. 2009. Effect of pH on germination of the endemic heather, *Erica andevalensis*. J. Aquat. Plant Manage. 47: 57-59.
- Vicient C. 2017. The effect of frequency-specific sound signals on the germination of maize seeds. BMC research notes. 10(1):323. <https://doi.org/10.1186/s13104-017-2643-4>
- Waskow A, Howling A and Furno I. 2021. Mechanisms of plasma-seed treatments as a potential seed processing technology. Front. Phys. 9:617345. doi: 10.3389/fphy.2021.617345.
- Wong K, Chew N, Low M, Tan M. 2023. Plasma-activated water: physicochemical properties, generation techniques, and applications. Processes. 1:2213.
- Zhou R, Li J, Zhou R, Zhang X, Yang S. 2019. Atmospheric-pressure plasma treated water for seed germination and seedling growth of mungbean and its sterilization effects on mung bean sprouts. Innov. Food Sci. Emer. Technol. 56: 36-44. doi: 10.1016/j.ifset.2018.08.006.
- Zulfiqar F. 2021. Effect of seed priming on horticultural crops. Scientia Hortic. 286: 110197.