Effects of different treatments of atmospheric pressure plasma on the germination and seedling development of soybean (*Glycine max*) seeds

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ABSTRACT

Soybean (*Glycine max*) is a major source of vegetable protein and an important agricultural commodity in the Philippines; however, its thick seed coat limits germination efficiency. This study investigated the effects of argon–nitrogen atmospheric pressure plasma on soybean seed germination and seedling growth through direct, indirect, and combined treatments. In direct treatment, seeds were exposed to plasma for 3–7 s, while indirect treatment used plasma-activated water (PAW) generated for 5–15 min. The combined method applied both direct plasma (3–7 s) and PAW (5 min). Optical emission spectroscopy (OES) confirmed the presence of reactive and excited argon, nitrogen, and trace oxygen species in the plasma. Scanning electron microscopy (SEM) revealed surface microcracks and wax removal after plasma exposure, while contact angle analysis showed increased hydrophilicity and water uptake with longer treatment times. PAW displayed reduced pH and elevated nitrate levels compared to the control. The combined treatment demonstrated the most pronounced and consistent improvements in germination potential by 22%, shoot length by 54–66%, shoot fresh weight by 31–43%, and shoot air-dried weight by 44–77%. In contrast, direct plasma treatment consistently enhanced root-related early growth performance, with increases of 16–20% in germination rate, 22–29% in root length, 40–50% in root fresh weight, and 31–44% in root air-dried weight. These findings highlight plasma technology as a promising approach to improve soybean seed germination and early growth.

INTRODUCTION

Soybean (*Glycine max*) is a widely utilized legume valued for its high protein content and vegetable oil (Porto et al. 2018). In the Philippines, it is considered a major imported agricultural commodity. Soybeans are consumed in various forms, including soy milk, soy sauce, tofu, coffee substitutes, and other food preparations. Beyond human consumption, they are also incorporated into animal feed for their protein content, while soybean oil is also processed for biodiesel production as a renewable energy source (Balanay et al. 2021). Despite its versatility and demand, local soybean production remains minimal and inconsistent. Consequently, the Philippines relies heavily on importation, sourcing about 99% of its annual consumption from major producers such as the USA, Brazil, and China, while domestic production accounts for only about 1% (Agcopra et al. 2018). To reduce the country's dependence on costly imports, import substitution has been suggested as a strategy to promote domestic soybean production and reduce reliance on imports. This approach could help farmers generate higher income while also creating additional job opportunities (Agcopra et al. 2018).

Soybean seeds have thick seed coats that comprise approximately 4–8% of seed weight. Water penetration usually occurs through cracks on the dorsal side of the seed, but the cuticle acts as an efficient barrier to water intake. This can delay germination, reduce germination percentage, and contribute to poor yield or seed dormancy (Ranathunge et al. 2010; Guragain et al. 2021). These challenges highlight the need for alternative approaches to enhance soybean establishment and yield (Guragain et al. 2021).

In recent years, non-thermal plasma (NTP) technology has gained considerable attention in fields such as sterilization, medicine, and agriculture. It generates reactive species, including ions, electrons, and neutral particles, and its chemistry can be modified by introducing different feed gases. These variations allow for the development of seed-treatment technologies that differ significantly from conventional methods (Domonkos et al. 2021). Due to its low operating temperature, NTP can be applied directly to seeds without causing thermal damage, enabling surface modification that enhances hydrophilicity, sterilizes seed surfaces, and reduces pathogen invasion in soil, ultimately improving

KEYWORDS

atmospheric pressure plasma, plasma-activated water, soybeans, germination, seedling development

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germination. Indirect treatment using plasma-activated water (PAW) also shows promise, with potential benefits for both seed germination and plant growth (Attri et al. 2020).

The effectiveness of NTP in enhancing seed germination has been demonstrated in several crops, including carrots and other seed types (Guragain et al. 2021). In soybeans, studies using direct NTP treatment through dielectric barrier discharge have also shown positive effects on germination (Ling et al. 2014). In the Philippines, the commonly available soybean varieties/genotypes are PSB Sy 2, PSB Sy 6, and PSB Sy 8. Among these, PSB Sy 2, also known as Tiwala 6, has been identified as the most adaptable variety under local conditions (Zabala 2020). Although numerous studies have reported the effectiveness of NTP and PAW for surface modification and germination improvement, limited research has focused on the potential of plasma surface technology as a pre-sowing treatment for PSB Sy 2 (Tiwala 6). Specifically, there is a lack of studies examining its role in enhancing hydrophilicity, improving water uptake, and accelerating germination. Moreover, only a few reports exist on the working parameters of NTP using an atmospheric pressure plasma jet system for soybean seed surface modification.

Therefore, this study investigated the effects of different atmospheric pressure plasma treatments such as direct, indirect, and combined treatments on the germination and seedling development of soybean seeds.

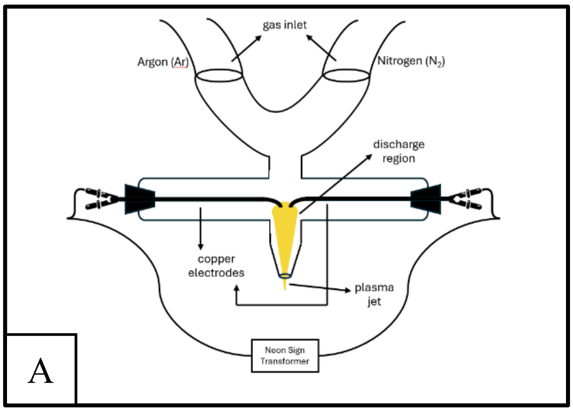
MATERIALS AND METHODS

Atmospheric Pressure Plasma Jet Setup

The study employed a modified atmospheric pressure plasma jet (APPJ) system, illustrated in Figure 1 (Malapit and Baculi 2022). The discharge unit consisted of two electrodes made of 99.95% pure copper, each 1.5 mm in diameter, positioned 5 mm apart, and enclosed within a customized glass chamber. The chamber featured a 2 mm nozzle outlet for the plasma plume and was sealed with rubber stoppers. Two gas inlets, located at the top of the chamber, were connected to argon (2.5 L/min) and nitrogen (5 L/min) cylinders, providing a combined volumetric flow rate of 1.25×10^{-4} m^3/s. Based on the continuity equation, the corresponding plume velocity was calculated to be 39.79 m/s.

Plasma generation was driven by a 450 W neon sign transformer, delivering an output of 15 kV at 30 mA, which was connected to the electrodes for PAW production. For direct treatment, the transformer output was regulated using a variable autotransformer (variac), enabling precise voltage adjustment. Under a variac input of 120 V, the corresponding output voltage was 7.8 kV. The relationship between variac input (x) and output voltage (y) was established through linear regression analysis of experimental measurements, yielding the following equation (Pineda et al. 2025)

$$y = 64.716x - 10.458$$



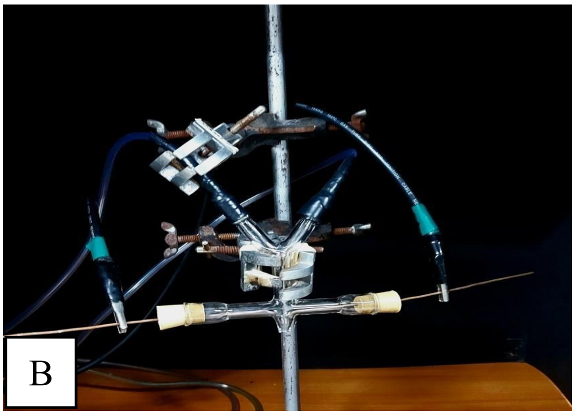


Figure 1: **(A)** Schematic diagram and **(B)** actual photo of the modified atmospheric pressure plasma jet (APPJ) setup

Plasma Discharge Characterization

Optical emission spectroscopy (OES) was performed to identify the dominant species present in the argon nitrogen plasma. An optical fiber cable was directed toward the generated plasma plume, and the corresponding spectral lines were recorded using Ocean View software through a spectrometer connected to a computer. The recorded emission spectra were further analyzed with Spectrum Analyzer 1.97 software to determine the electron temperature of the discharged plasma using Boltzmann plot. To confirm that the plasma was non-thermal (cold), the gas temperature was verified using a Cooper-Atkins K-type thermocouple.

Seed Material and Viability Test

PSB Sy 2 soybean seeds (Tiwala 6) were obtained from the Department of Agriculture–Cagayan Valley Research Center. A seed viability test was conducted to ensure only intact and viable seeds were used in the experiment. Seeds were placed in a 400 mL beaker containing distilled water, and those that sank to the bottom were presumed viable. The selected seeds were air-dried overnight on two layers of tissue paper before plasma exposure.

Atmospheric Pressure Plasma Treatments of Soybeans

Soybean seeds were subjected to three types of plasma treatments: direct, indirect, and a combination of both. For direct treatment, seeds were exposed to plasma for 3, 5, or 7 s, with the nozzle positioned 1 cm away from the seed surface. Only one side of each seed was treated, and the seeds were manually raster-scanned in circular motions to ensure uniform plasma exposure and to prevent localized overheating or damage. These treated seeds were then used for germination and seedling development tests. For indirect treatment, untreated seeds were germinated using PAW in place of distilled water. PAW was prepared by treating 200 mL of sterilized distilled water with plasma for 5, 10, or 15 min. Lastly, combined treatment involved the use of soybean seeds exposed to plasma (direct treatment) and subsequently germinated with PAW (5 min) throughout the germination and seedling development tests.

Experimental Design

The experiment followed a randomized complete block design with three replications to ensure reliability and accuracy of the results. Seeds were germinated in Petri dishes lined with two layers of filter paper, with 10 untreated or treated seeds placed per replicate and 10 mL of sterilized distilled water or PAW initially added. For the direct treatment, 10 mL of sterilized distilled water was supplied every other day to maintain adequate moisture. For the indirect and combined treatments, 10 mL of PAW was added every other day to sustain the moist environment required for continuous seed exposure. All samples were maintained at room temperature (25–27 °C).

This study was conducted under controlled laboratory conditions using a relatively small experimental unit of 10 seeds per Petri dish with three replications and focused only on early germination and seedling development over a 10-day period. Therefore, the findings may not fully represent long-term growth performance or field-level responses under natural agricultural conditions.

Germination and Seedling Development Evaluation

Germination was defined as the emergence of the radicle. Petri dishes were monitored every 24 h for 10 days to record germination potential and germination rate using the formulas below.

$$Germination\ potential\ (\%) = \frac{no. of\ seeds\ germinated\ in\ 3\ days}{total\ no. of\ seeds} \times 100$$

$$Germination\ rate\ (\%) = \frac{no. of\ seeds\ germinated\ in\ 7\ days}{total\ no. of\ seeds} \times 100$$

After 10 days, shoot and root lengths were also measured using a vernier caliper. Shoots were then separated from roots for biomass analysis. Fresh weight was measured immediately after sampling, while air-dried weight was determined after 24 h of air drying.

Seed Surface Morphology Characterization

Surface modification of untreated and plasma-treated seeds was examined using a Hitachi TM4000Plus scanning electron microscope (SEM) at the Philippine Science High School–Ilocos Sur. Imaging was conducted under the following conditions: accelerating voltage of 15 kV, magnification range of 30×–600×, and scale bars of 50 µm - 1 mm. SEM images of untreated and all treated samples were compared to observe physical changes induced by plasma treatment.

Seed Surface Wettability

Wettability of untreated and plasma-treated seeds was assessed through contact angle measurement. A 20–30 µL droplet of distilled water was placed on a relatively flat region of the seed surface using a micropipette, and the apparent contact angle (angle between the droplet and seed surface) was recorded. Droplet images were captured with a digital microscope and analyzed using the DropSnake plugin in ImageJ software to determine contact angles (Stalder et al. 2010; Schneider et al. 2012). Triplicate measurements were carried out to ensure accurate and reproducible results.

Seed Water Uptake

Water uptake of untreated and plasma-treated seeds was evaluated gravimetrically. Seed weights were recorded using an analytical balance prior to soaking in 50 mL centrifuge tubes containing distilled water for 2, 4, 6, 8, 16, and 24 h. At each interval, seeds were removed, blotted/air-dried on two layers of tissue paper for 2 h, and reweighed. Final seed water uptake was then calculated for each treatment parameter using the formula:

$$Water\ Uptake\ (\%) = \frac{W_t - W_0}{W_0} \times 100$$

where W_t is the seed weight after soaking and drying, and W_0 is the initial seed weight.

Plasma-Activated Water Characterization

Plasma-activated water (PAW) was characterized by measuring pH and nitrate concentrations. The pH of untreated and treated water samples was determined using a PASCO pH sensor. Nitrate concentrations were quantified using UV–Vis spectrophotometry at 220 nm. Calibration standards ranging from 0–7 ppm were prepared and used as references to calculate nitrate content in the samples. Distilled water was used as the blank prior to measurement.

Statistical Analyses

Statistical analyses were performed using the Statistical Tool for Agricultural Research (STAR) software. One-way analysis of variance (ANOVA) was conducted to determine whether significant differences existed among the treatment groups, because the experiment followed a randomized complete block design (RCBD). When significant differences were observed, Tukey's Honestly Significant Difference (HSD) or Least Significant Difference (LSD) test was used for multiple comparisons of means. All statistical analyses were evaluated at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Plasma Generation and Characterization

During plasma generation for indirect treatment, the argon–nitrogen feed gases ignited between the two copper electrodes under an applied voltage of 15 kV, producing a yellowish discharge. The observed emission color is consistent with the findings of Koch et al. (2017). The yellowish hue observed in the present study may be attributed to the combined emission of nitrogen and argon species, together with trace oxygen contributions from ambient air. The plasma was subsequently characterized using optical emission spectroscopy (OES) to determine the dominant active species present. Argon, nitrogen, and oxygen lines were selected from the Spectrum Analyzer software to refine the spectral analysis.

The wavelength–intensity profile (Figure 2) revealed characteristic emissions of singly-ionized argon, Ar II, at 336.174 nm and 374.691 nm; neutral nitrogen, N I, at 346.654 nm; singly-ionized nitrogen, N II, at 319.639 nm, 360.909 nm, and 392.464 nm; neutral oxygen, O I, at 405.477 nm; singly-ionized oxygen, O II, at 398.541 nm; and singly-ionized copper, Cu II, at 378.627 nm. The detection of these spectral lines confirms that the generated discharge is an argon–nitrogen plasma comprising mixtures of ionized argon, nitrogen, and oxygen species.

Furthermore, analysis of the emission spectrum indicated electron and gas temperatures of 13,606 K and 371 K, respectively. The substantial disparity between these values supports the non-thermal nature of the plasma, characterized by electrons possessing significantly higher energy than the bulk gas.

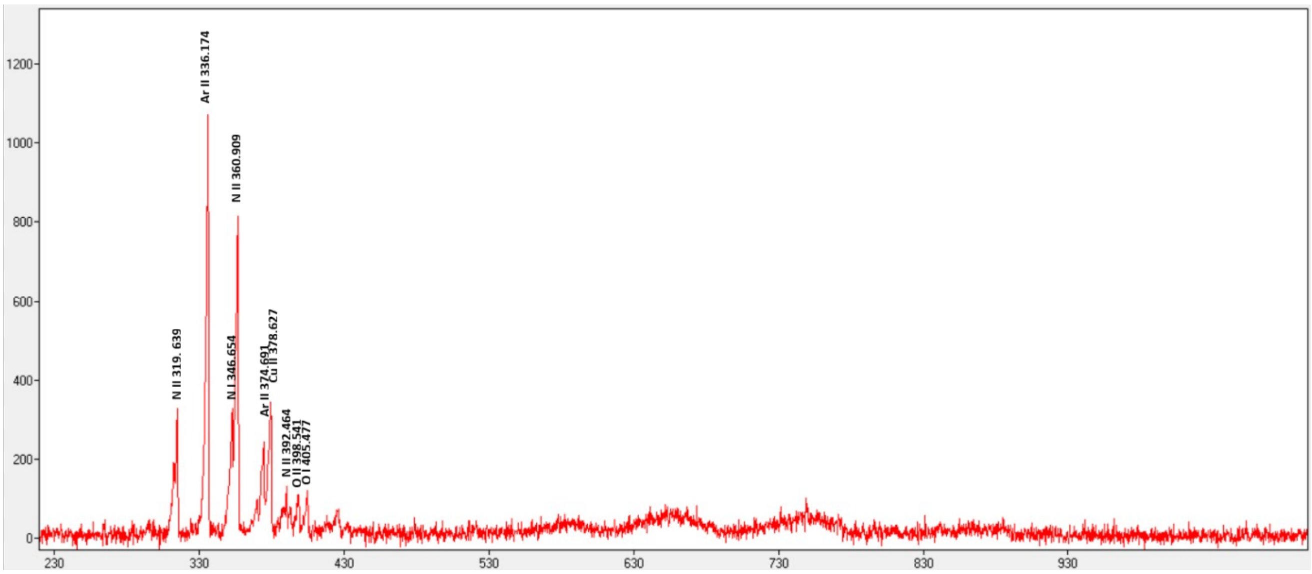


Figure 2: Optical emission spectrum of argon-nitrogen plasma for indirect treatment

Seed Surface Morphology Characterization

The surface modification of untreated and plasma-treated soybean seeds was examined using scanning electron microscopy (SEM). The SEM images show the outer seed coat surface, particularly on the dorsal side of the seed, where natural cracks are typically concentrated. The dorsal side is the side containing the hilum, which is the scar where the seed was attached to the pod. At low magnification (30×), the seed surface morphology, as shown in Figure 3, revealed that increasing plasma exposure time enhanced the formation of microcracks, attributable to plasma etching.

ImageJ software was used to measure the widths of the observed microcracks. No detectable microcracks were observed in the control and 3 s plasma-treated seeds. In contrast, the 5 s plasma-treated seeds exhibited microcracks of approximately 0.323 mm width, while the 7 s plasma-treated seeds showed narrower microcracks of 0.176 mm width. Although the measured crack width was greater after 5 s than after 7 s, the 7 s treatment appeared to produce longer cracks.

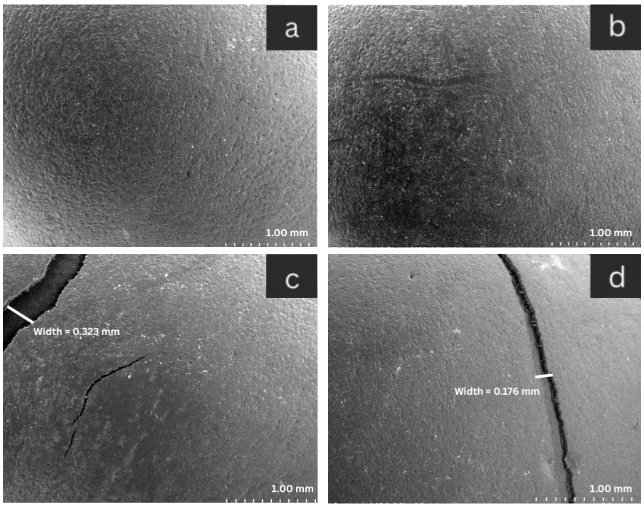


Figure 3: SEM images using 30× magnification. (**A**) Untreated seeds (**B**) 3 s plasma-treated (**C**) 5 s plasma-treated (**D**) 7 s plasma-treated

Soybean seeds thick coat acts as a barrier to water uptake, with the outer cuticle in particular limiting imbibition. Water penetration typically occurs on the dorsal side of the seed, where natural cracks are concentrated. However, the restricted permeability of the coat delays imbibition, often prolonging germination time and reducing germination efficiency. In some cases, inadequate water absorption results in seed dormancy, contributing to poor overall yield.

Plasma technology has been widely demonstrated to improve surface hydrophilicity across various materials, including textiles and seeds (Peran et al. 2020; Lotfy, 2017). In the present study, the interaction of reactive nitrogen species (RNS) and reactive oxygen species (ROS) generated during plasma treatment facilitated surface etching. The etching process removed epicuticular wax layers and induced microcracks on the seed coat, thereby enhancing permeability. This modification promotes faster water uptake and potentially improves germination performance (Priatama et al. 2022).

At higher magnification (500–600×), SEM micrographs shown in Figure 4 provided further evidence of progressive structural changes with increasing treatment duration. Smaller microcracks were observed in the 5 and 7 s plasma-treated seeds, indicating enhanced surface alteration compared to the untreated samples. Plasma bombardment by reactive species produced both physical etching and chemical oxidation, disrupting the hydrocarbon matrix of the waxy cuticle. The combined action of RNS and ROS may have degraded surface lipids, effectively reducing hydrophobicity and facilitating water passage through the seed coat barrier (Priatama et al. 2022).

These results indicate that plasma treatment effectively alters the soybean seed coat, creating structural modifications that are expected to improve water absorption and accelerate germination.

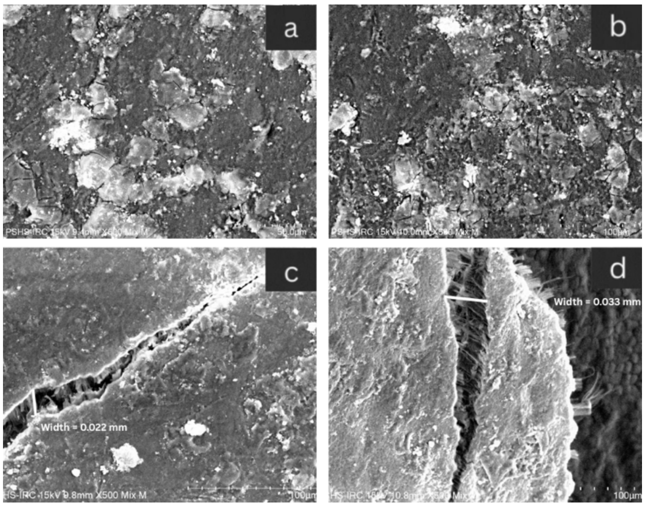


Figure 4: SEM images using 500x-600x magnification. (**A**) Untreated seeds (**B**) 3 s plasma-treated (**C**) 5 s plasma-treated (**D**) 7 s plasma-treated

Apparent Contact Angle Measurement

The apparent contact angle was measured to evaluate changes in seed surface wettability following plasma treatment. Measurements were performed on a relatively flat seed surface using water droplets, and each trial was repeated three times to ensure accuracy. As shown in Figure 5, the contact angle decreased progressively with increasing treatment time. Untreated soybean seeds exhibited an average contact angle of 100.798 ± 0.196°, indicating a hydrophobic surface. After plasma exposure, the average contact angle decreased to 81.069 ± 0.977° (3 s), 63.303 ± 0.707° (5 s), and 34.020 ± 0.981° (7 s).

Wettability describes the ability of a liquid to spread across a solid surface. Surfaces are considered hydrophobic when the apparent contact angle exceeds 90°, whereas contact angles below 90° indicate hydrophilicity (Huhtamäki et al. 2018). Apparent contact angle is defined as the angle formed between the curved surface of the seed and the tangent to the droplet at the point of contact (Bormashenko et al. 2012). In this study, plasma treatment progressively reduced the apparent contact angle, confirming a transition from hydrophobic to hydrophilic behavior.

The improvement in surface wettability can be attributed to the action of RONS generated during plasma etching. These species likely disrupted the epicuticular wax layer and created microcracks, thereby enhancing water permeability. This surface modification provides pathways for water penetration into the seed coat, a critical factor in the early stages of germination. These findings are consistent with the observations of Ling et al. (2014) who also reported enhanced hydrophilicity of plasma-treated seeds. The apparent contact angle measurements confirm that plasma treatment effectively enhances the hydrophilicity of soybean seeds, facilitating water absorption and potentially improving germination performance.

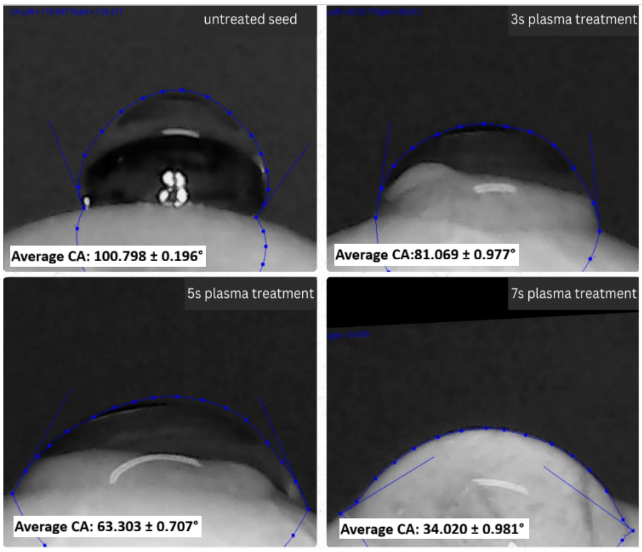


Figure 5: Contact angle measurement of soybean seeds. (**A**) untreated seeds (**B**) 3 s plasma-treated (**C**) 5 s plasma-treated (**D**) 7 s plasma-treated

Seed Water Uptake

Figure 6 presents the water uptake of soybean seeds measured over 24 h. It shows that during the first 2 h, plasma-treated seeds exhibited greater weight gain compared to untreated seeds. After 4 h, the treated seeds continued to absorb water at a markedly higher rate, while untreated seeds showed only minimal uptake. Prolonged treatment time further enhanced the rate of water absorption. Direct plasma treatment exposed the soybean seed surface to electrons, ions, and reactive radicals, resulting in etching and partial erosion of the seed coat. This modification alters the originally hydrophobic outer layer, increasing its hydrophilicity and thereby enhancing water uptake (Alves et al. 2016). As soaking progressed, untreated seeds exhibited a gradual increase in water uptake, whereas treated seeds absorbed water more rapidly in the early hours, a factor critical to the onset of germination (Alves et al. 2016). The enhanced uptake can be attributed to plasma-induced physical modification of the seed coat and chemical oxidation caused by reactive oxygen and nitrogen species (RONS). These processes removed epicuticular waxes and created microchannels that facilitated water penetration.

An inverse relationship was observed between the apparent contact angle and seed water uptake. Plasma treatment reduced the contact angle, thereby increasing hydrophilicity, which corresponded with enhanced water absorption. After 24 h, however, the water uptake values of treated and untreated seeds converged, as sufficient time allowed water to diffuse into both seed types (Holc et al. 2022). These findings indicate that plasma treatment accelerates water absorption during the early soaking period, which is vital for improving germination efficiency and seedling establishment.

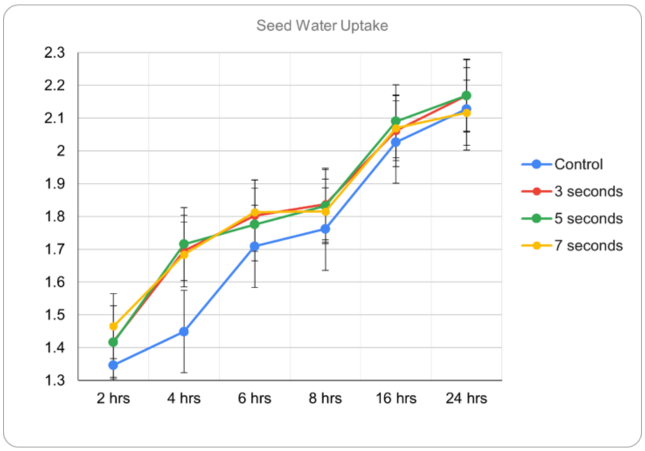


Figure 6: Water uptake of untreated and plasma-treated soybean seeds over 24 h

Plasma-Activated Water Characterization

The plasma-activated water (PAW) was characterized by measuring pH and nitrate concentration as presented in Table 1, which shows the decrease in pH and increase in nitrate concentration with treatment time.

Untreated sterilized distilled water exhibited an initial pH of 6.71 ± 0.225. Following plasma treatment, the pH progressively declined to 5.86 ± 0.057 after 5 min, 5.50 ± 0.200 after 10 min, and 5.16 ± 0.115 after 15 min. The observed acidification of PAW is attributed to the interaction of water with reactive chemical species generated during plasma discharge. Previous studies have reported the formation of hydrogen peroxide and nitric acid in PAW, which have been reported to play crucial roles in enhancing seed germination. Consistent with earlier reports, the reduction in pH was correlated with increasing treatment time, although pH values may also vary depending on the feed gas composition used for plasma generation (Thirumdas et al. 2018).

The determination of nitrate levels followed Method B of the Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 2017). The nitrate concentrations of PAW treated for 5, 10, and 15 min were found to be 1.34 mg/L, 1.39 mg/L, and 1.50 mg/L, respectively. These results align with the findings of Vichiansan et al. (2023) confirming that increasing plasma exposure enhances nitrate generation. The elevated nitrate levels are likely due to interactions between nitrogen ions formed during plasma discharge and water molecules, leading to the formation of nitrate and related reactive nitrogen species.

The combined decrease in pH and increase in nitrate concentration indicate that PAW becomes chemically enriched with reactive nitrogen species during treatment, suggesting its potential role as a biostimulant in promoting seed germination and early seedling development.

Table 1: Changes in pH and nitrate levels of plasma-activated water with treatment time

PAW Treatment Time (min)	pH	Nitrate concentration* (mg/L)
0	6.71 ± 0.225	0
5	5.86 ± 0.057	1.34
10	5.50 ± 0.200	1.39
15	5.16 ± 0.115	1.50

*Measured only once

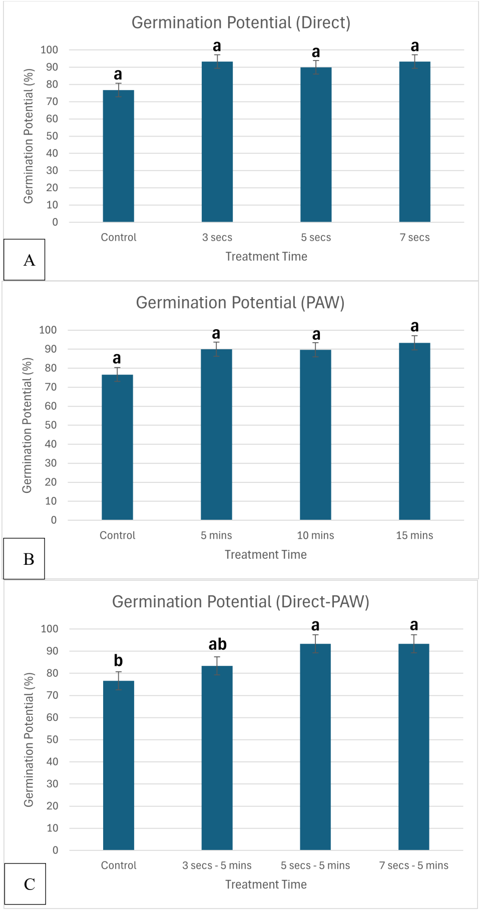
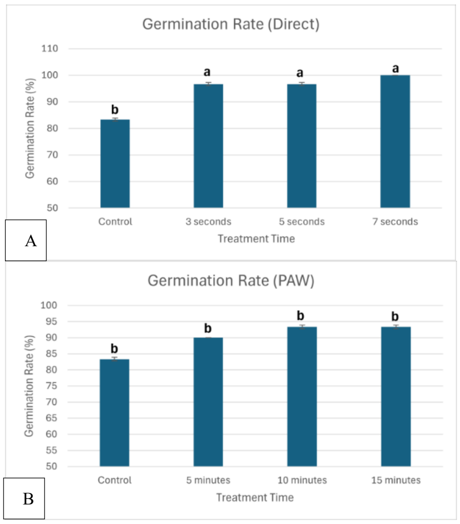


Figure 7: Effects of (**A**) Direct (**B**) Indirect, and (**C**) Combined Plasma Treatments on the Germination Potential of Soybeans. Error bars indicate standard error (n=3). Means with different lowercase letters indicate statistically significant differences among treatment groups at the 95% confidence level



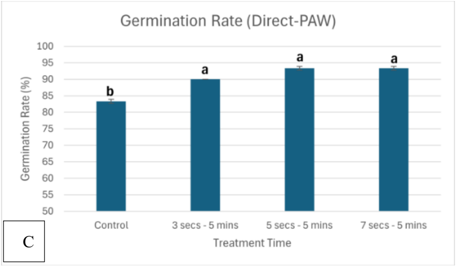


Figure 8: Effects of (**A**) Direct (**B**) Indirect, and (**C**) Combined Plasma Treatments on the Germination Rate of Soybeans. Error bars indicate standard error (n=3). Means with different lowercase letters indicate statistically significant differences among treatment groups at the 95% confidence level

Role of Plasma Treatments on the Germination of Soybeans

Germination is a physiological process in which seeds shift from dormancy to active metabolic activity, culminating in seedling emergence. This transition is regulated by a combination of intrinsic and environmental factors that determine whether dormancy is maintained or germination is initiated. The timing of germination is a critical determinant of plant development and crop performance, as both premature germination (pre-harvest sprouting) and delayed germination can adversely impact yield and uniformity (Shu et al. 2015). The effects of various plasma treatments on the germination potential and rate of soybeans are illustrated in Figures 7 and 8.

Figure 7 shows the germination potential of soybeans treated using direct, indirect and combined plasma treatments. It shows that the control (untreated) has the lowest germination potential across all treatments. The result shows that direct and indirect treatments can enhance their germination potential to some extent but cannot improve it significantly within the experimental treatment durations. The same result was observed by the study conducted by Ling et al. (2014) in their study on the effects of cold plasma treatment on the germination of soybeans where no significant difference was observed on the germination potential after plasma treatment. However, it is apparent from the figure that the combined treatment led to a significant improvement of about 22% increase in the germination potential, specifically the treatments 5 s-5 min and 7 s-5 min, which indicates that the combination of both direct and indirect treatments could directly impact the potential of soybean seeds to germinate more readily than untreated seeds.

On the other hand, Figure 8 shows the effects of various plasma treatments on the germination rate of soybeans. The use of plasma-activated water alone did not significantly improve the germination rate consistent with the germination potential results, which is likely due to its low nitrate levels and possibly limited concentrations of other reactive species even after 15 min of plasma treatment. However, it can be seen from the figure that both direct and combined plasma treatments significantly enhanced the germination rate by 8–20%.

Plasma treatment has been shown to enhance germination across various seeds, including wheat, rice, and carrot. In wheat, atmospheric pressure plasma improved germination under optimal conditions of 3 min for direct treatment and 15 min for plasma-activated water (Chalise et al. 2023). Rice seeds exposed to dielectric barrier discharge air plasma and plasma-activated mist exhibited 36.73% and 50.4% increases in germination potential, respectively, with corresponding gains in germination rate and index (El et al. 2023). Carrot seeds treated with non-thermal plasma also demonstrated higher germination percentage and vigor index (Guragain et al. 2021).

A comparative investigation of direct plasma treatment and PAW irrigation demonstrated that both approaches significantly influenced water absorption, seed germination, and root development, effects that were primarily attributed to plasma-induced RONS. The study further underscored that variations in plasma operating parameters and feedstock gas composition modulated the concentrations of reactive species in both seed- and water-based treatments, thereby shaping the physiological responses of soybean (Engeling et al. 2018).

Evidence from several reports indicates that both direct seed exposure to plasma and irrigation with PAW promote soybean germination and seedling vigor. These enhancements have been associated with improved water uptake, elevated reactive species levels, and induced physiological modifications in seedlings. Nevertheless, plasma does not always exert positive effects on germination. Some studies have observed negligible or even reduced germination rates, which appear to depend on seed type, treatment conditions, and plasma parameters, suggesting variability in plant responses. The overall efficacy of plasma-based treatments is contingent upon multiple factors, including discharge power, treatment duration, feed gas type, and the resultant chemical characteristics of PAW (Guragain et al. 2021; Ling et al. 2014; Engeling et al. 2018; Chen et al. 2023; Sayahi et al. 2024; Barjasteh et al. 2023).

Table 2: Effects of Different Plasma Treatments on the Shoot Length and Shoot Biomass of Soybeans

Plasma Treatments	Time of Exposure	Seedling Development Parameters*		
		Shoot Length (cm)	Shoot Fresh Weight (g)	Shoot Air-Dried Weight (g)
Direct	Control (Untreated)	9.06 ± 0.76a	5.94 ± 0.37b	1.16 ± 0.06c
	3 s	11.86 ± 1.41a	7.87 ± 0.38a	1.56 ± 0.25b
	5 s	11.47 ± 2.25a	8.44 ± 0.22a	1.94 ± 0.22a
	7 s	12.39 ± 1.78a	8.70 ± 0.85a	1.86 ± 0.22ab
Indirect (PAW)	Control (Untreated)	9.06 ± 0.76c	5.94 ± 0.37b	1.16 ± 0.06b
	5 min	11.64 ± 0.51b	7.82 ± 0.69a	1.79 ± 0.12a
	10 min	12.97 ± 2.18ab	8.15 ± 0.62a	1.98 ± 0.26a
	15 min	14.62 ± 0.53a	7.82 ± 0.69a	1.85 ± 0.33a
Combined (Direct + PAW)	Control (Untreated)	9.06 ± 0.76b	5.94 ± 0.37b	1.16 ± 0.06b
	3 s + 5 min	15.06 ± 0.92a	8.41 ± 0.31a	2.05 ± 0.23a
	5 s + 5 min	13.94 ± 1.36a	7.77 ± 0.95a	1.71 ± 0.30a
	7 s + 5 min	13.97 ± 1.59a	8.48 ± 0.38a	1.88 ± 0.17a

*Means with different lowercase letters indicate statistically significant differences within each treatment group only at the 95% confidence level

Role of Plasma Treatments on the Shoot Length and Shoot Biomass of Soybeans

The effects of various plasma treatments on soybean shoot length and biomass are presented in Table 2. The data indicate that direct plasma treatment of soybean seeds did not result in a significant increase in shoot length; however, it markedly enhanced shoot biomass. The fresh weight of shoots from plasma-treated seeds ranged from 7.87 to 8.70 g, representing a 33% to 46% increase compared with the control. Similarly, the air-dried weight of shoots ranged from 1.56 to 1.94 g, corresponding to a 34% to 67% increase over the control. Notably, variations in treatment duration did not exert a pronounced effect on shoot length and biomass.

Direct plasma treatments influence soybean shoot length and biomass through mechanisms associated with seed surface modification and physiological activation. Evidence shows that pre-sowing exposure of soybean seeds to non-thermal plasma, particularly under optimized conditions can markedly enhance shoot growth. In a study conducted by Ling et al. (2014), shoot length and shoot dry weight increased by approximately 13.77% and 21.95%, respectively, relative to untreated seeds, indicating improved early vegetative development following plasma application.

Cold plasma treatment has also been reported to promote shoot elongation and biomass accumulation in several seed types. In tomato, atmospheric pressure dielectric barrier discharge (DBD) plasma combined with seed flipping markedly increased shoot length and vigor index relative to controls (Murali and Sumathi 2025). In wheat, both plasma jet and DBD treatments enhanced shoot length, air-dried weight, root growth, and germination rates, with improvements attributed to greater seed surface hydrophilicity and water uptake (Velichko et al. 2019). In cereal grains, cold plasma treatment has been shown to enhance shoot length, shoot weight, root length, and overall biomass (Pourbagher et al. 2024). In pea and lentil, treatments with dielectric barrier discharge and pin electrode reactor systems increased shoot dry weight while also improving root traits and nodulation, suggesting a wider positive influence on plant growth, including shoot biomass (Abeysingha et al. 2024).

In contrast, indirect treatment using PAW significantly enhanced shoot length. The control reached 9.06 cm, whereas the treated seeds grew to 11.64–14.62 cm, representing a 28% to 61% increase. The PAW generated with the longest treatment duration (15 min) produced the greatest improvement in shoot length, likely due to the higher concentration of reactive species. For example, this treatment yielded the highest nitrate levels, as shown in Table 1. Consistent trends were observed in shoot biomass. The fresh weight of shoots from PAW-treated seeds was 32% to 37% greater than the control, while air-dried weight increased by 54% to 71%. However, variations in treatment time did not significantly influence shoot fresh or dry biomass. Consequently, the shortest treatment duration (5 min) was selected for the combined plasma treatment.

PAW has been reported to enhance soybean shoot growth and biomass. Studies show that PAW treatment improves early vegetative parameters, particularly shoot length. For example, soybean seeds exposed to PAW generated with N_2/O_2 mixtures produced nearly twice the plant length of seeds treated with distilled water, with the strongest effects observed at gas ratios of 80/20 and 50/50. These improvements were linked to higher nitrate concentrations and electrical conductivity in PAW, indicating that reactive species formed under specific gas conditions play a key role in promoting shoot development (Chen et al. 2023).

The combination of direct and indirect (PAW) plasma treatment also resulted in significant improvements in both shoot length and shoot biomass. Shoot length increased by 54% to 66%, while fresh and air-dried weights rose by 31% to 43% and 47% to 77%, respectively. Similar to the indirect treatment, variations in treatment duration did not exert a significant influence on either shoot length or biomass. Overall, the combined treatments produced the most pronounced enhancement in both parameters.

The observed increase in shoot biomass has been attributed to improvements in germination performance and vigor indices, both of which are positively modulated by plasma treatment. These enhancements are linked to greater water uptake and a reduction in seed surface contact angle, reflecting improved wettability and imbibition that facilitate more effective seedling establishment (Ling et al. 2014; Ďurčányová et al. 2023). Furthermore, plasma treatment has been shown to accelerate the mobilization of seed reserves, thereby supplying additional nutrients to support shoot growth during the initial stages of development (Ling et al. 2014).

Furthermore, the effects of plasma treatment on soybean growth vary depending on the working gas employed. Nitrogen (N_2) and helium (He) plasmas have been shown to be more effective than argon (Ar), oxygen (O_2), or hydrogen (H_2) plasmas in promoting shoot elongation and biomass accumulation. These treatments enhanced plant height and fresh weight across different developmental stages, suggesting a sustained positive influence on shoot growth (Viliya et al. 2024). In the present study, argon was used as one of the working gases because it is more available and less costly than helium, yet it still produced promising results in enhancing both shoot length and biomass.

When applied with appropriate gas combinations and optimized power settings, plasma treatments can enhance soybean shoot length and biomass by modifying seed surface properties, promoting water uptake, and stimulating nutrient mobilization. These effects collectively contribute to more vigorous early growth and greater biomass accumulation (Ling et al. 2014; Ďurčányová et al. 2023; Viliya et al. 2024).

Table 3: Effects of Different Plasma Treatments on the Root Length and Root Biomass of Soybeans

Plasma Treatments	Time of Exposure	Seedling Development Parameters*		
		Root Length (cm)	Root Fresh Weight (g)	Root Air-Dried Weight (g)
Direct	Control (Untreated)	8.67 ± 0.65c	2.09 ± 0.12b	0.16 ± 0.01b
	3 s	11.15 ± 0.63a	3.14 ± 0.05a	0.21 ± 0.01a
	5 s	9.58 ± 0.85bc	2.93 ± 0.14a	0.23 ± 0.00a
	7 s	10.56 ± 0.34ab	3.11 ± 0.53a	0.21 ± 0.04a
Indirect (PAW)	Control (Untreated)	8.67 ± 0.65a	2.09 ± 0.12b	0.16 ± 0.01b
	5 min	8.43 ± 0.50a	2.89 ± 0.50b	0.22 ± 0.04b
	10 min	7.53 ± 0.94b	2.28 ± 0.80b	0.18 ± 0.07b
	15 min	7.67 ± 0.61b	2.47 ± 0.15b	0.19 ± 0.01b
Combined (Direct + PAW)	Control (Untreated)	8.67 ± 0.65a	2.09 ± 0.12c	0.16 ± 0.01b
	3 s + 5 min	8.76 ± 0.95a	2.93 ± 0.07a	0.19 ± 0.04a
	5 s + 5 min	7.87 ± 0.81a	2.41 ± 0.23bc	0.20 ± 0.01a
	7 s + 5 min	7.31 ± 0.06a	2.66 ± 0.22ab	0.21 ± 0.01a

*Means with different lowercase letters indicate statistically significant differences within each treatment group only at the 95% confidence level

Role of Plasma Treatments on the Root Length and Root Biomass of Soybeans

The effects of different plasma treatments on soybean root length and biomass are shown in Table 3. The results indicate that direct plasma treatment of soybean seeds produced significant increases in both root length and biomass. The root length of the control was 8.67 cm, whereas the treated seeds attained 10.56–11.15 cm, representing a 22% to 29% increase. The fresh weight of roots from plasma-treated seeds ranged from 2.93 to 3.14 g, corresponding to a 40% to 50% increase relative to the control. Likewise, the air-dried weight ranged from 0.21 to 0.23 g, reflecting a 31% to 44% increase over the control. Variations in treatment duration did not significantly influence root biomass. This result was consistent with the study conducted by Ling et al. (2014) in which they reported that the cold plasma treatment of soybean seeds significantly increased root length and dry weight, with improvements of about 21.42% and 27.51%, respectively, relative to untreated controls. In addition, cold plasma treatment with dielectric barrier discharge and pin electrode reactors increased root dry weight, length, volume, and surface area of pea and lentil, indicating enhanced root biomass (Abeysingha et al. 2024). Moreover, plasma exposure generally promoted root length and weight in cereal grains including wheat, rice, corn, and barley, supporting improved growth efficiency and biomass accumulation (Pourbagher et al. 2024). These findings highlight the stimulatory effect of direct plasma on root development, suggesting a preferential enhancement of root growth.

In contrast, indirect treatment exhibited either negligible or negative effects on root length and biomass. For example, PAW generated with a 5-min treatment did not significantly affect root length, whereas longer treatment durations (10 and 15 min) reduced root length by 12% to 13%. Likewise, the fresh and air-dried weights of root biomass in the control did not differ significantly from those of the PAW-treated groups. As treatment duration showed no significant influence on root biomass, the shortest duration (5 min), which also had a negligible effect on the root length was selected for the combined plasma treatment.

While PAW has been consistently reported to promote shoot growth and biomass in soybeans, as also demonstrated in the present study, its effects on root length may be negative under longer exposure durations, whereas its impact on root biomass seems negligible. This is consistent with the study of Jiresova et al. (Jirešová et al. 2022) in which PAW treatment improved germination, shoot length, and both fresh and dry shoot weights of wheat grains, yet root length and the root-to-shoot ratio exhibited slight reductions. These findings suggest that although PAW can positively influence shoot development and overall biomass accumulation, its impact on root parameters may be limited or mildly adverse.

On the other hand, the combination of direct plasma treatment and PAW did not produce significant improvements in root length. However, root biomass was notably affected, with fresh and air-dried weights increasing by 27% to 40% and 19% to 31%, respectively. Overall, among the three treatments, direct plasma application yielded the most pronounced enhancement in root length and biomass. The observed improvements in root length and biomass may be linked to enhanced water uptake and modifications in seed surface properties, including reduced contact angle, which facilitates better hydration and supports root growth (Ling et al. 2014).

CONCLUSION

This study demonstrates the potential of argon–nitrogen atmospheric pressure plasma as an innovative and potentially sustainable technology to improve soybean seed germination and early growth. The combined treatment proved most effective for enhancing germination potential and shoot development, while direct plasma exposure provided consistent benefits for germination rate and root growth. These improvements are primarily attributed to plasma-induced physicochemical modifications, such as surface restructuring, enhanced water uptake, and microcrack formation. In contrast, PAW contributes through chemical effects, including the generation of reactive nitrogen species, nitrate enrichment, and acidification, which play supportive roles that enhances overall performance of the combined treatment.

Beyond soybeans, these findings suggest that plasma technology could be applied to other crops as a seed priming strategy to promote uniform germination, stronger seedling establishment, and with potential implications for yield improvement. Future studies involving field validation, long-term growth assessments, and molecular-level mechanistic investigations will be essential to fully elucidate the agricultural potential of plasma-based seed treatments and to strengthen their practical applicability under real-world farming conditions.

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CONFLICT OF INTEREST

The authors declare no competing interests.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

J.P.L. Bernardo: Conceptualization; Methodology; Formal analysis; Investigation; Writing—original draft; Writing—review & editing. **M.M.M. Rubio:** Conceptualization; Methodology; Formal analysis; Supervision; Writing—review & editing. **G.M. Malapit:** Conceptualization; Methodology; Supervision.

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