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

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ABSTRACT: The goal of this study is to explore how water treated using plasma affects the germination and growth of soybean and wheat seedlings. Deionized water (DIW) was exposed to a gliding arc discharge (GAD) for 5 and 10 min to create plasma-activated water. DIW revealed significant changes in physical properties as well as chemical parameters after its treatment with plasma. The germination rate (growth metrics, such as shoot/seedling length, imbibition rate of seeds, and vigor indices), increased when plasma-treated water was used for irrigation purposes. In conclusion, when plasma-treated water is used for irrigation, a better result yield in germination is acquired.

KEY WORDS: plasma agriculture, deionized water, gliding arc discharge, plasma-activated water, reactive oxygen and nitrogen species, soybean, wheat

I. INTRODUCTION

With the swift increase of the world population, the demand for global food has seen a dramatic increase due to factors such as less agricultural area, lower germination percentage, loss of seed survivability, as well as seeds getting contaminated during operations of transport, conditioning, grading, storage, and packaging conducted postharvest. These contamination in seeds slows down the germination rate. It has been the greatest challenge for developing countries like Nepal to produce safe and sufficient food of higher quality.

Along with the interdisciplinary approaches, cold plasma treatment is now identified as a viable solution for enhancing the seed sprouts and subsequent plant growth for crops such as radish, wheat, tomato, and soybean.[1–9] Apart from being advantageous for

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agricultural use, enhancement of the sprouting rate of seeds through nonthermal plasma techniques is beneficial for the food processing industry.[10–12] It has generally been accepted that, by boosting seed germination and plant cultivation rates, the global demand may be efficiently satisfied.[13,14] Different chemicals as well as physical methods have been used to fight against the problems. However, the major call-in question is to search for a sole solution that is economical. In order to tackle the problems of improving food production while ensuring food security, we should concentrate on the improvement of plasma-activated water (PAW) use.[8,15–20]

Wheat (*Triticum aestivum*) is one of the most widely cultivated crops, with ~ 700 million tons harvested each year for human consumption and animal feed.[21] Similarly, soybean [*Glycine max* (L.) Merrill] is a major worldwide oilseed crop, with production worth 347 million metric tons in 2017–2018.[6] Increasing populations and growing family wealth in developing and emerging nations have fueled steady increases in worldwide wheat and soybean consumption.[21,22] Hence, it is necessary to advance the techniques of crop production and enhance the germination of wheat and soybean seeds. It has also been proven that PAW possesses remarkable biotic activity that finds application for agricultural and medicinal purposes.[15,19,20,23,24]

PAW can also be used in agriculture to enhance the rate of seed sprouting and significant growth of the sprouts, inhibit the growth of pathogens, and treat fungus-infected sprouts.[25,26] In general, PAW is generated by arching and/or the application of a gliding arc discharge (GAD) on the surface of water. The plasma discharge has a direct effect on the seed coating and may impact the cells inside the seeds. Seeds treated in air plasma are susceptible to interactions with electrons, ions, reactive species, and UV radiation produced in the discharge.

The worthwhile results of plasma treatment arise from the synergistic effect of reactive neutral species, charged species (electrons, ions), electric fields, and ultraviolet radiation created in the discharge.[27] Long-lived reactive nitrogen-oxygen species like NO_3^-, NO_2^-, H_2O_2, O_3 and are generated in PAW.[24] Plasma treatment affects the metabolic process of plant development by causing alterations on the seed coat and allowing radicals to enter into the seed.[28–30] Most of the researchers reported that the germination rate of seed as well as the plant's propagation will be increased by irrigating them with plasma-treated water.[15,17,31–33] According to Naumova et al.,[34] PAW improved germinability of the seeds by 50%. Similarly, Møller et al.[35] reported that the seeds irrigated with water containing reactive oxygen species increases seed germination by breaking seed dormancy.

Many researchers found that length of stem and root of a plant were increased by adding PAW instead of normal water. Park et al.[36] found that the length of the alfalfa root and stem grew to 6.2 and 5 cm in PAW, respectively, in comparison to 1 cm in the controlled sample. Kučerová et al.[37] discovered that wheat plants irrigated with PAW generated using transient spark discharge have greater photosynthetic pigments and soluble proteins compared to normal. Than et al.[38] found that PAW treatments had a favorable influence on the growth characteristics of *Lactuca sativa* L. seedlings, including stem and root length, leaf weight, leaf area, and chlorophyll content, as well as seedling vigor. Alves et al.[39] discovered that *Erythrina velutina* seeds irrigated with PAW had a higher rate of germination and vigor than seeds irrigated with untreated water. Shaer et al.[40] observed that higher germination rates of wheat seedlings were seen when the seeds were irrigated by indirect wet PAW and plasma-activated mist in comparison to the application of a dry direct atmospheric pressure plasma jet to the seeds. Similarly, various researchers found that using PAW to irrigate soybean seeds increased the rate of germination and subsequent plant growth.[41,42]

Water activated using nonthermal PAW can remove or halt the growth of a range of bacteria; this might make the water useful as a fertilizer in addition to its antimicrobial characteristics. The synergistic effect resulting from a low pH and a strong positive oxidation-reduction potential is thought to be the source of PAW's bactericidal activity.[8,36,43–45] PAW is rising to be an eco-friendly and preferable option for biotechnological applications in agro-industry due to its distinct metabolic activity and environmental and economic benefits.[46,47] The effects of PAW on wheat (*T. aestivum*) and soybean (*G. max*) seed germination and its possible influence on seedling growth were investigated in this study.

II. EXPERIMENTAL SECTION

Figure 1 illustrates the empirical setup for treating the water. Two divergent aluminum electrodes (8.0 × 2.5 × 0.3 cm) are the main component around which the setup is built. The two electrodes are housed in a rectangular polycarbonate vessel (15 × 15 × 15 cm). A hole of 4 mm in diameter is bored on the top face allowing gas to flow inside the chamber. The electrodes are separated by a minimum of 3 mm. To treat water for irrigation, a Petri dish is placed 4 mm at the bottom of border of the electrodes. A 9 kV sinewave voltage running at a 50 Hz frequency was used to create the discharge between two diverging electrodes. A high-voltage probe (PINTEK HVP-28HF) was used to measure the discharge voltage, and an oscilloscope probe was used to measure the

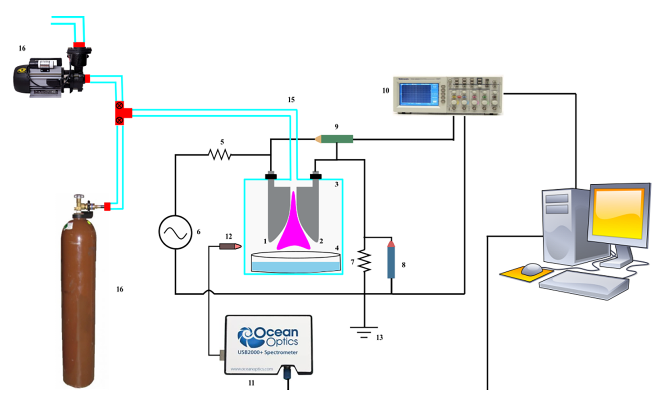

FIG. 1: Schematic diagram of the setup used for water treatment. 1,2: Electrodes, 3: Polycarbonate frame, 4: Petri dish with water, 5 Ballast resistor (1.7 MΩ), 6: Transformer, 7: Shunt resistor (10 KΩ), 8: Current probe, 9: Voltage probe, 10: Oscilloscope, 11: Spectrometer, 12: Optical fiber, 13: Earthing, 14: Computer interfacing, 15: Gas inlet, and 16: Gas cylinder/pump.

discharge current over a shunt resistance of 10 kΩ. The variation of current and voltage with time were documented using a digital storage oscilloscope (Tektronix TDS 2002) and processed for visualization.

A GAD was used on 50 mL of deionized water (DIW) for 5 and 10 min to create PAW. Water was stored in sterile reagent bottles after treatment before being used in physical, chemical, and analytical studies. pH and conductivity were measured with a standard multiparameter probe (Lutron, WA-2015) while UV-visible spectrophotometric technique (UV-1800, Shimadzu) was used to evaluate the chemical parameters in accordance with established protocols.[48]

A. Exploration of Germination Parameters

Fifty seeds of soybean and wheat were kept in a Petri dish filled with cotton for three repeated experiments. The seeds were then sprinkled with an equal amount of DIW and PAW consistently. Germination is defined to be a period when the seedling starts to sprout from the seed surface. Numerous parameters for germination were studied using the formula reported by various experimenters.[49–52]

B. Assessment of Water Uptake and Growth Characteristics

Fifty seeds of soybean and wheat were taken in three repetitions. Weight of the seeds were measured with an electronic scale (MG124Ai, Bel instruments) prior to soaking them in water (t) and hourly (t, $t + 1$, $t + 2$, $t + 3$, $t + 4$) after that, following a conventional formula for water uptake assessment.[6,53] Seedling lengths were estimated using a software, ImageJ version 1.53e on the tenth day after germination. After delicately removing seedlings from the Petri dish without any damage, seedling length was measured. Similarly, the vigor index was calculated according to the methods provided by various research scholars.[6,54]

C. Statistical Analysis

At least three replications were performed for all the experiments and mean ± standard deviation was used to demonstrate the result. One-way variance analysis was used to calculate the substantial difference in the mean of the physicochemical parameters of water, germination parameters, and seedlings length, and Tukey's multiple comparison test was conducted using Graph Pad Prism 8.0.2. Different letters in same column and graph indicate a noted difference in mean values at $p < 0.05$.

D. Electrical Signal Analysis

Figure 2 demonstrates the current–voltage signal of the GAD at 9 kV generated in air. The sawtooth nature of voltage, the waveform is seen while the current waveform is sinusoidal in nature.

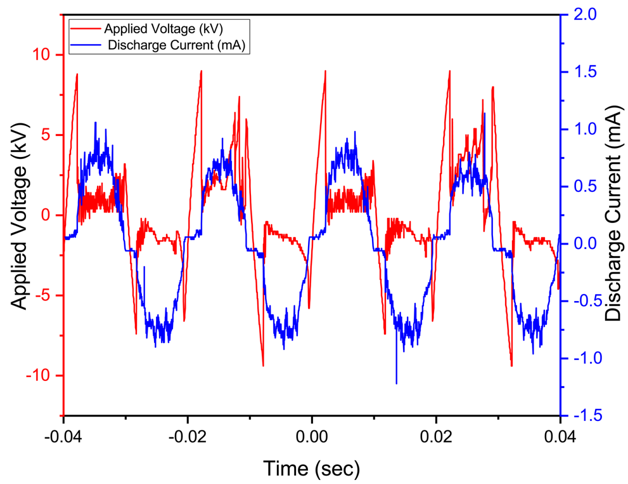

FIG. 2: Voltage-current signal of the GAD plasma in air using 50 Hz frequency

Integrating the voltage and current over time provides the power dissipated as follows:[55,56]

$$P = \frac{1}{T}\int_0^T I(t)V(t)\,dt \tag{1}$$

where $I(t)$ and $V(t)$ are the current and voltage values, respectively, for a period T. Using Eq. (1), power dissipated in the discharge was estimated to be 3.36 W.

Electron density is one of the most important parameters to determine the characteristics of plasma species. The following equation calculates the electron density:[57]

$$n_e = \frac{J_{av}}{e\mu_e E} \tag{2}$$

where E is the electric field, μ_e is the electron mobility, e is the electronic charge, and J_{av} represents average current density. For GAD plasma produced at 9 kV, electron mobility is calculated to be 408.8 cm^2/Vs with the help of Bolsig + software. After plugging these values into Eq. (2), we calculated electron density (n_e) to be 4.43×10^8 cm^{-3}.

E. Physical and Chemical Specifications

Exposure of GAD plasma in water produces reactive oxygen and nitrogen species (RONs) in it which is the signature of PAW.[24] After 10 min of treatment with plasma, the amount of ammonia, nitrites, and nitrates in PAW rose, reaching 4.79 ± 0.16, 0.53 ± 0.03, and 2.60 ± 0.08 mg/L, respectively (Table 1). These stable reactive RONS species are suspected for playing a key role in seed dormancy and germination control.[15,33,41,58]

Similarly, when discharge time increased, the pH of PAW notably decreased. After 10 min of plasma activation, the pH of the treated water decreased to 4.45 ± 0.05 in comparison to 6.40 for DIW. Water acidification is seen during plasma discharge because of the formation of peroxynitrous acid, HNO_2 and HNO_3, as reported by earlier studies.[8,59,60] Electrical conductivity (EC) usually identifies active ions present in a liquid and measures the liquid's ability to transport an electrical current.[19,24,61] There was a significant increment of EC in PAW samples as plasma activation time increased, reaching 58.6 ± 1.60 μS/cm at 10 min, pointing out the generation of active ions in PAW.

F. Influence on the Water Uptake of Soybean and Wheat Seeds due to PAW

Absorption of water is paramount for seed sprouting because it encourages the seed coat to widen and soften.[62] With a prolonged discharge treatment, PAW greatly increased the water soaking rate of soybean and wheat seeds, as shown in Fig. 3(a) and 3(B). When compared to seeds immersed in DIW, the imbibition rate of seeds soaked with plasma-treated water (soybean and wheat) rose by 22.87 ± 0.2 and 36.61 ± 0.4%, respectively, after 5 h of soaking. In several studies, data from a scanning electron microscope indicated a drastically changed structure on the seed coat of PAW-treated seeds. Seed coats may be chaffed resulting from the RONS present in high concentration in the water treated with plasma, assisting in moisture and mineral absorption.[18,63]

G. Impact of PAW on the Germination of Soybean and Wheat Seeds

From Table 2, we noted that seeds irrigated by plasma-treated water gain better sprouting potential compared to the seeds irrigated using DI water. On the sixth day, the final germination percentage of the seeds, i.e., soybean and wheat, irrigated using (5 and 10 min) of treated DI water increased by 32.67 and 43.57% and 13.15 and 23.68%, respectively, compared to the control seedlings. Seeds of soybean and wheat irrigated using PAW sprout faster than seeds irrigated with DIW, suggesting that more PAW-irrigated seeds germinate. DIW- and PAW-irrigated seeds have a substantial difference in the mean germination rate. Furthermore, it was noted that there is a significant change in the variability in the germination process and synchrony of the sprouting process between DIW- and PAW-irrigated seedlings of soybean and wheat.

TABLE 1: Effect of GAD on the physicochemical parameters of DIW

Liquid	Treatment time	Nitrates (mg/L)	Nitrites (mg/L)	Ammonia (mg/L)	pH	Electrical conductivity (µS/cm)
DIW	Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
	5 Min	1.43 ± 0.12^{b}	0.45 ± 0.03^{b}	1.05 ± 0.07^{b}	4.78 ± 0.06^{b}	48.8 ± 1.40^{b}
	10 Min	4.79 ± 0.16^{a}	0.53 ± 0.03^{a}	2.60 ± 0.08^{a}	4.45 ± 0.05^{a}	58.6 ± 1.60^{a}

Different letter (a–c) in same column indicates the significant different in mean values at $p < 0.05$, one-way ANOVA, *post hoc* Tukey, multiple comparison test.

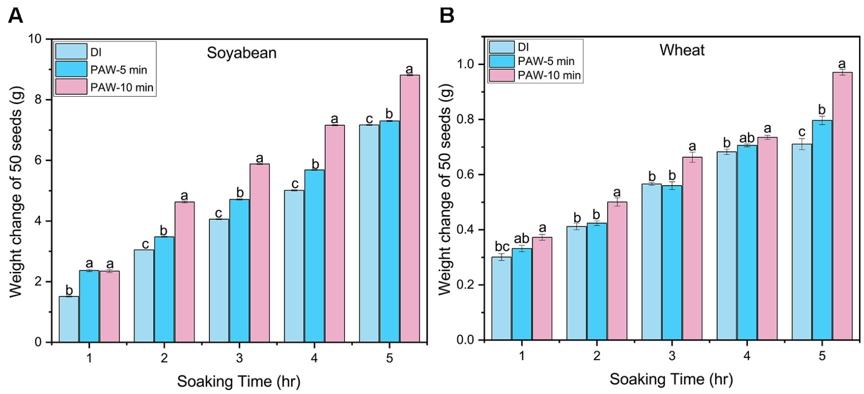

FIG. 3: Imbibition rate of (A) soybean and (B) wheat seeds irrigated using DIW and PAW for different soaking periods. Separate letters denote a notable contrast between group ($p < 0.05$).

As shown in Table 3, the coefficient of the velocity of germination time differs significantly between DIW-irrigated soybean seeds and seeds watered with DI water treated for 10 min. However, there is no noticeable difference in CV_t value between soybean seedlings irrigated with DIW and DIW water treated for 5 min. In the case of wheat, however, we found a substantial change in CV_t value. Furthermore, compared to seeds irrigated with DI water, seeds irrigated with 5- and 10-min treated DIW exhibited increases in CVG of 6.13 and 11.50% in soybean and 8.05 and 5.41% in wheat, respectively. The coefficient of germination velocity (CVG) measures the rate of seeds germination.[50]

In comparison to seeds irrigated with DIW, the germination index (GI) of seeds doused with 5 minutes, and 10 minutes treated DIW rose by 40.13% and 61.21% in the case of soybean and 17.29 and 31.27% in the case of wheat. A higher GI score points towards a higher percentage and rate of germination.[64] Furthermore, we observed a notable change in the MDG values comparing the seedlings that were irrigated using DIW and PAW.

Similarly, seeds irrigated with 5- and 10-min treated DIW had an 86.31 and 124.14% increase in germination value (G-value) in soybean and a 25.73 and 51.41% increase in wheat, respectively, when compared to seeds irrigated with DIW. From Table 4 we noted that seed irrigated using PAW germinates considerably faster than the seed irrigated using DI water.

The production and metabolism of reactive species are consistently believed to be in dynamic equilibrium.[58,59] By interfering with the abscisic acid/gibberellic acid signaling pathways, reactive species (RONs) generated in plasma-treated water may operate as positive signal molecules, reducing the seed latency period and accelerating seed sprouting, resulting in an enhanced seed germination.[65,66] The metabolic process of plant growth is influenced by long-lived reactive nitrogen-oxygen species produced in PAW, which cause changes on the seed surface and allow radicals to enter the seed, resulting in an improved germination.[33,59,63,67]

TABLE 2: Effect of plasma-treated water on the sprouting parameters for soybean and wheat seeds

Seeds	treatment time	Final germination percentage (%)	Mean germination time (day)	Mean germination rate (day)$^{-1}$	Uncertainty of germination process (U) (bit)	Synchrony of germination process (Z)
Soybean	Control	67.33 ± 1.15^{c}	4.75 ± 0.02^{a}	0.21 ± 0.0011^{c}	1.32 ± 0.02^{c}	0.49 ± 0.01^{a}
	5 min	89.33 ± 1.15^{b}	4.48 ± 0.01^{b}	0.22 ± 0.0011^{b}	1.68 ± 0.01^{b}	0.34 ± 0.00^{b}
	10 min	96.67 ± 1.15^{a}	4.26 ± 0.01^{c}	0.23 ± 0.0011^{a}	1.70 ± 0.01^{a}	0.32 ± 0.00^{c}
Wheat	Control	76.00 ± 2.00^{c}	4.71 ± 0.02^{a}	0.21 ± 0.0011^{b}	2.26 ± 0.01^{c}	0.19 ± 0.00^{c}
	5 min	86.00 ± 2.00^{b}	4.56 ± 0.01^{b}	0.21 ± 0.0011^{b}	2.23 ± 0.02^{b}	0.20 ± 0.00^{b}
	10 min	94.00 ± 2.00^{a}	4.46 ± 0.01^{c}	0.22 ± 0.0011^{a}	2.20 ± 0.01^{a}	0.21 ± 0.00^{a}

Different letter (a–c) in same column indicates the significant different in mean values at $p < 0.05$, one-way ANOVA, *post hoc* Tukey, multiple comparison test.

TABLE 3: Effect of PAW on the germination parameters for soybean and wheat seeds

Seeds	Treatment time	Coefficient of variation of germination time (CV_t) (%)	Coefficient of velocity of germination (CVG) (%)	Germination index (GI) (day)	Mean Daily germination percent (MDG) (%)	Germination value (G-value)
Soybean	Control	18.61 ± 0.61^{b}	21.04 ± 0.13^{c}	7.40 ± 0.19^{c}	2.49 ± 0.04^{c}	29.60 ± 1.07^{c}
	5 min	18.65 ± 0.34^{b}	22.33 ± 0.09^{b}	10.37 ± 0.19^{b}	3.30 ± 0.04^{b}	55.15 ± 1.47^{b}
	10 min	21.57 ± 0.19^{a}	23.46 ± 0.08^{a}	11.93 ± 0.19^{a}	3.58 ± 0.04^{a}	66.36 ± 1.62^{a}
Wheat	Control	27.87 ± 0.21^{c}	21.23 ± 0.14^{c}	8.73 ± 0.19^{c}	2.81 ± 0.07^{c}	33.22 ± 1.35^{c}
	5 min	28.83 ± 0.24^{b}	22.94 ± 0.14^{b}	10.24 ± 0.19^{b}	3.18 ± 0.07^{b}	41.77 ± 1.54^{b}
	10 min	29.81 ± 0.25^{a}	22.38 ± 0.14^{a}	11.46 ± 0.19^{a}	3.48 ± 0.07^{a}	50.30 ± 1.69^{a}

Different letter (a–c) in same column indicates the significant different in mean values at $p < 0.05$, one-way ANOVA, *post hoc* Tukey, multiple comparison test.

TABLE 4: Variation of time to T_{10}, T_{25}, T_{75}, and T_{90} germination between DIW- and PAW-irrigated seeds

Seeds	Treatment time	T_{10} (%)	T_{25} (%)	T_{50} (%)	T_{75} (%)	T_{90} (%)
Soybean	Control	2.60 ± 0.05^{a}	4.08 ± 0.01^{a}	4.44 ± 0.01^{a}	4.80 ± 0.01^{a}	5.16 ± 0.01^{a}
	5 min	2.67 ± 0.05^{a}	3.34 ± 0.03^{b}	4.12 ± 0.01^{b}	4.63 ± 0.01^{b}	4.93 ± 0.00^{b}
	10 min	2.36 ± 0.01^{b}	2.90 ± 0.02^{c}	3.76 ± 0.02^{c}	4.54 ± 0.01^{c}	4.87 ± 0.00^{c}
Wheat	Control	2.42 ± 0.01^{a}	3.05 ± 0.02^{a}	4.18 ± 0.02^{a}	5.27 ± 0.07^{a}	5.99 ± 0.07^{a}
	5 min	2.36 ± 0.00^{b}	2.90 ± 0.02^{b}	3.92 ± 0.02^{b}	5.11 ± 0.06^{b}	5.92 ± 0.06^{a}
	10 min	2.31 ± 0.00^{c}	2.78 ± 0.01^{c}	3.75 ± 0.02^{c}	5.10 ± 0.04^{b}	5.88 ± 0.05^{a}

Different letter (a–c) in same column indicates the significant different in mean values at $p < 0.05$, one-way ANOVA, *post hoc* Tukey, multiple comparison test.

H. Influence of PAW Treatment on Seedling Development

Photographs of soybean and wheat sprouts germinated with DIW and PAW are shown in Fig. 4(A)–4(D).

Shoot, root, and seedling length measurements of soybean and wheat seedlings were taken after 10 days of sowing [Fig. 5(A)–5(B)]. We found that the seedlings irrigated with 5- and 10-min treated DI water boosted seedling length by 28.83 and 42.83%, respectively, and shoot length by 50.88 and 61.7%, respectively, as compared to DIW-irrigated soybean seedlings.

Similarly, in the case of wheat, seedling length improved by 8.2 and 38.6%; and shoot length rose by 12 and 62.31% when irrigated using DIW for 5 and 10 min, respectively. No significant differences in root length were seen in PAW- and DIW-irrigated seedlings. The increase in growth parameters might be due to rise in concentrations of reactive RONS stable for a longer time in PAW.[8,17,68,69]

III. CALCULATIONS FOR VIGOR INDEX

Figure 6 show that the Vigor Index I of soybean and wheat significantly increased by (70.92 and 105.06%) and (22.43 and 71.44%) in the case of 5- and 10-min DIW-irrigated seedlings, respectively, when collated with seedlings irrigated by DIW. Similarly, seedlings irrigated with plasma-treated water showed an increase in Vigor Index II. The

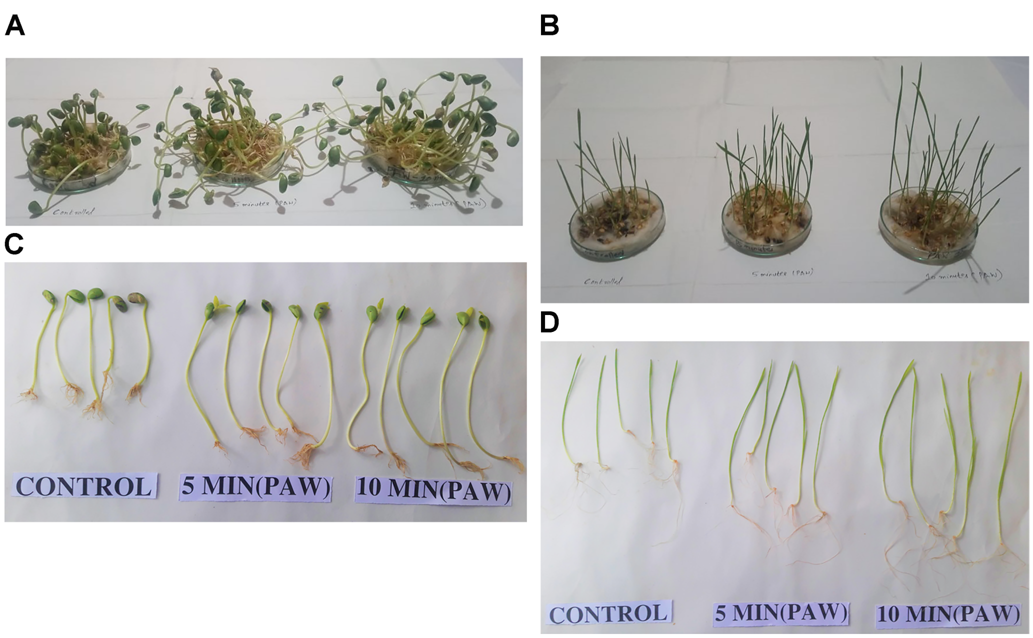

FIG. 4: (A, C) soybean and (B, D) wheat seed irrigated by DIW and PAW

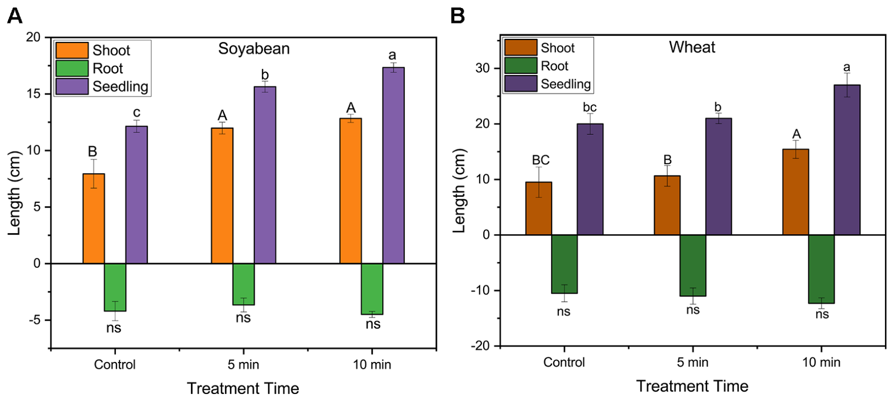

FIG. 5: Effect of DIW and PAW on the seedling length of (A) soybean and (B) wheat. Different letters denote significant difference between group ($p < 0.05$).

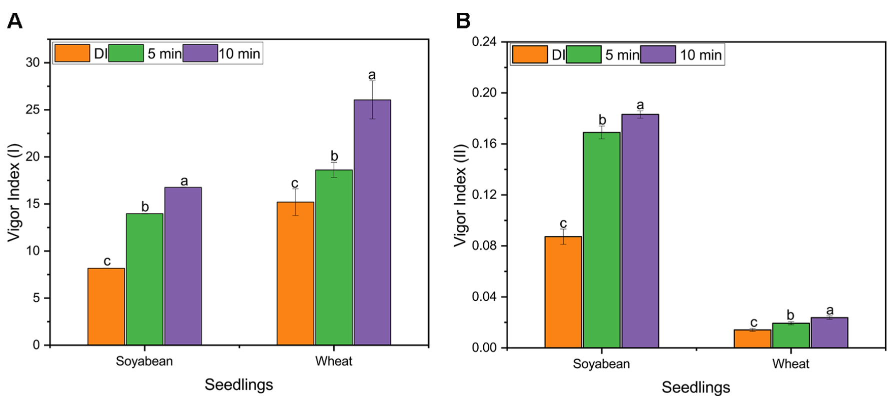

FIG. 6: Effect of DIW and PAW on seed's (A) vigor index (I) and (B) vigor index (II). Different letters denote significant difference between group ($p < 0.05$).

reaction of the RONs in PAW with the seed surface may lead to higher water and nutrient absorption, as well as increased germination percentage, GI, and vigor index.[17,33,34,70]

IV. CONCLUSIONS

In this work, reactive nitrogen and oxygen species were produced in deionized water using a 50 Hz gliding arc discharge. The electron density was calculated to be 4.43×10^8 cm^{-3}. The discharge's power consumption was calculated to be 3.36 W. When the treatment time was increased, there was increase in amounts of nitrites, nitrates, and ammonia in DIW. We conclude that the PAW enhanced the water absorption rate, germination rate, vigor index, and development of soybean and wheat seedlings as compared to DIW.

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