



Enhancing Germination and Early Growth of Millets (*Sorghum bicolor* L. and *Pennisetum glaucum* L.) Using Plasma-Activated Water

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Abstract

The application of non-thermal plasma in agriculture has emerged as an eco-friendly technology to enhance seed vigor and crop productivity. In this study, the effects of plasma activated water (PAW) generated through atmospheric pressure gliding arc discharge (GAD) plasma were evaluated on two staple millets, Jowar (*Sorghum bicolor* L.) and Bajra (*Pennisetum glaucum* L.). PAW treatments of varying activation times (5, 10, 15, and 20 min) were tested against a control (untreated water). Physicochemical analysis revealed a decrease in pH (from ~5.8 to ~3.7), along with significant increases in electrical conductivity, oxidation - reduction potential, and nitrate concentration (up to ~240 mg L⁻¹) with increasing activation time. Biological assays demonstrated that PAW significantly enhanced seed performance and early growth in both crops. For *Sorghum bicolor* L., 10 min PAW treatment was identified as the optimum, resulting in an increase in germination from 92% (control) to 98%, a reduction in mean germination time, and ~25–30% improvement in shoot length. For *Pennisetum glaucum* L., 15 min PAW treatment was optimal, yielding germination enhancement from 89% to 97%, ~32% higher water imbibition, and up to ~35–38% increases in shoot and root lengths. Chlorophyll content also improved significantly, with increases of ~19% in sorghum (10 min PAW) and ~23% in pearl millet (15 min PAW). Results suggest that PAW can serve as a sustainable pre-sowing and irrigation technology to improve seed vigor and early growth of millets, contributing to food security and resilience in semi-arid agroecosystems.

Keywords: Non - thermal plasma, Plasma agronomy, RONS mediated effects, Germination kinetics, Seedling vigor index, Nitrogen enrichment, Stress-resilient crops, Sustainable crop enhancement.

1. Introduction

Millets, especially sorghum (*Sorghum bicolor* L.) and pearl millet (*Pennisetum glaucum* L.), are among the most climate resilient cereal crops globally, widely grown in arid and semi-arid regions of Asia, Africa, and parts of South America. Their resilience to drought, heat, and poor soils makes them central to food and nutritional security in marginal farming systems [1-2]. These grains are not only sources of calories, but also provide essential micronutrients, dietary fiber, and antioxidants, which are critical in regions where malnutrition persists [3-4].

Despite their inherent resilience, millet productivity is often constrained not only by environmental factors but also by initial seed quality, which is strongly influenced by storage conditions, seed aging, and physiological maturity at harvest. Prolonged storage under suboptimal temperature and humidity can lead to reduced viability, loss of membrane integrity, enzyme inactivation, and oxidative damage, ultimately resulting in poor germination performance. In addition, issues such as seed dormancy, mechanical damage during post - harvest handling, and variability in seed lots further contribute to inconsistent field emergence. One of the primary bottlenecks is poor seed vigor, which encompasses slow or uneven germination, low seedling emergence, and weak early growth [2]. Additionally, nutrient stress, both in terms of soil fertility (e.g., low available nitrogen, phosphorus) and water availability, compounds the problem of stand establishment. Environmental variability, including erratic rainfall, high temperatures, and saline soils, further challenge early developmental stages, compromising final yield potential [1, 5]. Traditional approaches to ameliorate seed germination and vigor, such as chemical coatings,

synthetic seed treatments, or priming with salts (e.g., KNO_3 , CaCl_2), have shown benefits in millets under stress. However, concerns over environmental effects, cost, and residue limit their scalability or acceptance among smallholder farmers. Thus, there is a growing interest in sustainable, non-chemical methods that can improve seed performance, particularly under resource limited conditions.

In recent years, non-thermal plasma and plasma activated water (PAW) have emerged as promising tools in seed science. Non-thermal plasma, generated under atmospheric pressure (including dielectric barrier discharge [DBD] or gliding arc systems), produces reactive oxygen and nitrogen species (RONS), UV light, charged particles, and electric fields, which can modify seed coat chemistry, improve wettability, and stimulate metabolic activity [6-8]. PAW, in particular, incorporates many of these reactive species in water, reducing pH, increasing oxidation-reduction potential, and introducing bioavailable nitrogen forms such as nitrate and nitrite, which may act both as signaling molecules and mild fertilizers [2,6,9].

Specific studies in millets are now emerging. For example, Mohandoss, Mohan, Balasubramaniyan et al. [2] reported that pearl millet seeds treated with non-thermal DBD plasma showed enhanced germination rate (~20-30% higher) and plant growth. Their work also demonstrated that PAW generated by treating water under plasma decreased pH, increased nitrate concentrations, and helped change seed surface properties (e.g., reduced water contact angle) leading to higher imbibition and germination. Similarly, cold plasma treatment of pearl millet grain and flour [3,5] improved storage stability, reduced antinutritional compounds, and preserved bioactive compounds, further arguing for broader utility of plasma technologies in millet value chains.

Pre-sowing hydration-based treatments are widely employed to improve germination performance in millets. Among these, seed priming (including hydropriming and nutrient priming) is a well established technique in which seeds are partially hydrated to initiate metabolic activity, followed by re-drying to their original moisture content prior to sowing. This approach has been shown to enhance germination index, seed vigour, and yield under stress conditions, such as salinity in pearl millet [1]. However, it is important to distinguish priming from direct seed soaking or irrigation based treatments, where seeds are exposed to water (or treated solutions) and sown without a drying step. In the present context, the use of plasma-activated water (PAW) primarily aligns with a hydration/irrigation based treatment rather than conventional seed priming, as no post-treatment drying was performed. Additionally, physiological seed maturity plays a critical role in germination behaviour. Seeds harvested before full maturity often exhibit reduced germinability, indicating that seed quality and vigour are governed not only by external treatments but also by intrinsic developmental parameters.

Taken together, these findings suggest that PAW and non-thermal plasma treatment may address several constraints in millet cultivation, enhancing seed vigor, improving early growth, and potentially contributing to better yield and nutritional quality. However, gaps remain: relatively few studies have combined PAW with pot or field trials for millets, and optimal activation times or plasma exposure durations are not yet well defined. This study therefore investigates the effects of varying PAW activation times on germination, seedling vigor, root and shoot growth in sorghum and pearl millet under both controlled (Petri dish) and pot culture conditions. We aim to identify the best treatment durations for achieving significant improvements, to contribute to sustainable technological options for millet growers in climate-stressed environments.

2. Materials and methods

2.1 Experimental setup for plasma generation

The experimental setup for producing and characterizing atmospheric pressure gliding arc discharge (GAD) was designed following standard plasma agronomy protocols. Figure 1 illustrates the schematic of the arrangement. The discharge was generated using ambient air at a controlled flow rate of 8 Lmin^{-1} , which was directed between two copper electrodes, each 60 mm long and 2.5 mm in diameter. The electrode separation was adjusted from 1.5 mm at the inlet to 5 mm at the outlet to allow a stable gliding arc profile. The discharge voltage was supplied by a DC high-voltage source, adjustable between 0 and 10 kV.

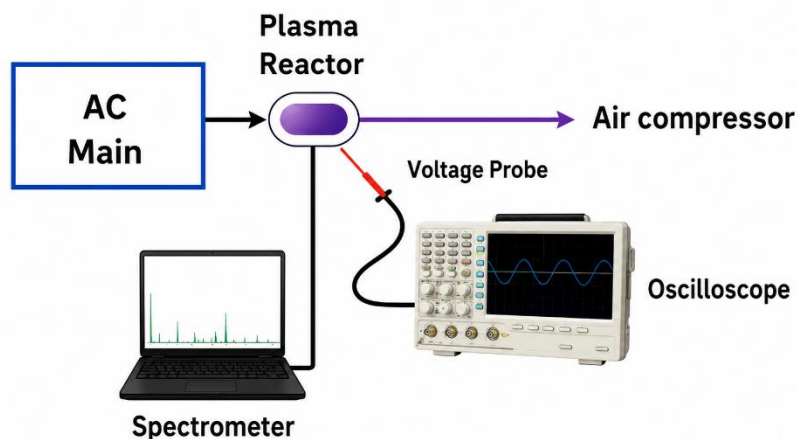


Figure 1 Schematic diagram of experimental arrangement

The electrical characterization of the plasma was performed using a 1000× high-voltage probe coupled to a digital oscilloscope to monitor discharge voltage. A 100 Ω series resistor was connected to the ground electrode, and the current was measured using a probe. Optical emission spectra (OES) of the discharge were recorded using a high-resolution spectrometer, with an optical fiber placed 8 mm below the electrode tips. Each spectrum represented the average of 30 scans at an integration time of 300 ms, ensuring accurate identification of plasma-generated reactive species.

2.2 Preparation of Plasma-Activated Water (PAW)

Plasma-activated water (PAW) was produced by treating 50 mL of deionized water positioned 10 mm below the arc region of the electrodes. Treatment durations were set at 5, 10, 15, and 20 minutes, hereafter referred to as 5 PAW, 10 PAW, 15 PAW, and 20 PAW, respectively. Untreated deionized water served as the control (0 PAW). The treated water was immediately used for seed priming and irrigation to preserve the short-lived reactive species.

The physicochemical properties of PAW were analyzed to quantify treatment induced changes. pH, total dissolved solids (TDS), electrical conductivity (EC), and oxidation - reduction potential (ORP) were measured using a multiparameter water quality tester. Calibration was performed using standard buffer solutions of pH 4.0, 6.5, and 9.1. Nitrite (NO_2^-) and nitrate (NO_3^-) concentrations were determined semi-quantitatively using colorimetric test strips with >99% efficiency, following established protocols [2,6]. Since reactive nitrogen species are central to the biological activity of PAW, additional precautions were taken to ensure the reliability of the strip based measurements. The test strips used in this study possess a manufacturer reported detection efficiency greater than 99% within the operational concentration range. Measurements were conducted in triplicate under identical experimental conditions, and color development was recorded within the recommended reaction time to minimize observational variability. Further, the obtained nitrate and nitrite trends were found to be consistent with corresponding changes in electrical conductivity and oxidation - reduction potential, supporting the validity of the measurements. Although ion chromatography or UV-Vis spectrophotometric analysis would provide higher analytical precision, the present semi-quantitative approach was considered suitable for comparative evaluation of relative changes in reactive nitrogen species with increasing plasma exposure duration.

2.3 Seed Material

Certified seeds of sorghum (*Sorghum bicolor* L. var. CSV 21F) and pearl millet (*Pennisetum glaucum* L. var. HHB 67 Improved) were procured from licensed vendor and verified for purity and viability. Seeds were surface-sterilized using 1% sodium hypochlorite for 5 minutes, followed by three washes with sterile distilled water, and then air-dried under shade for 2 hours.

For treatment, seeds were subjected to a pre-sowing hydration (soaking) process using plasma - activated water (PAW) corresponding to different activation times (5, 10, 15, and 20 min). Seeds were soaked in PAW for a fixed duration (specify duration, e.g., 6 - 12 h if applicable) and then immediately used for germination and pot experiments without re-drying to their initial moisture content. Therefore, the procedure followed in this study corresponds to a pre-sowing treatment rather than conventional seed priming, where re-drying is a necessary step.

2.4 Experimental Conditions

2.4.1 Germination Assay

To evaluate seed germination under laboratory conditions, the experiment was conducted using a completely randomized design (CRD). Each treatment consisted of five replications, with 10 seeds per experimental unit, resulting in a total of 50 seeds per treatment. The seeds were placed on Whatman No. 1 filter paper and moistened with 10 mL of plasma-activated water (PAW) corresponding to different activation times or with untreated distilled water (control). The seeds were directly exposed to PAW during germination, without any prior priming or re-drying step. Each dish was moistened with 10 mL of PAW or control water and incubated in a controlled growth chamber (25 ± 2 °C; 60–70% RH; 12 h light/12 h dark). Each treatment was replicated 5 times, resulting in 50 seeds per treatment group. Germination was monitored on a daily basis, considering a seed germinated when the radicle attained a length of 2 mm. To evaluate germination performance, several parameters were calculated, including final germination percentage (FGP), mean germination time (MGT), mean germination rate (MGR), germination synchrony (Z), coefficient of velocity of germination (CVG), and uncertainty of germination (U). These indices were determined using standard formulas as described by Ranal and Santana [10].

2.4.2 Pot Culture Experiment

To simulate soil based growth, pot experiments were conducted using plastic glasses as pots (8 cm diameter, 15 cm depth), each filled with 0.5 kg of a soil mixture containing loam, sand, and vermicompost in a 2:1:1 ratio. The pH of the soil mix was measured at 7.5. Each pot was sown with 5 seeds, and thinning was done after germination to maintain 2 uniform seedlings per pot. For each treatment (0, 5, 10, 15, and 20 PAW), 5 pots were maintained, amounting to 25 seedlings per treatment group.

Plants were irrigated daily with 20 mL of PAW or control water. The experiment was conducted under natural light conditions (28–32 °C day, 20–24 °C night; 55–75% RH).

2.5 Measurement of Growth Parameters

2.5.1 Water Imbibition

The water imbibition rate was measured by soaking 100 seeds in 20 mL of PAW/control water for 24 h. Initial (W_i) and final (W_f) seed weights were recorded, and imbibition was calculated as:

$$\text{Water imbibition rate (in \%)} = \frac{W_f - W_i}{W_i} \times 100$$

2.5.2 Germination Indices

Final Germination Percentage (FGP):

$$\text{FGP} = (\text{Number of seeds germinated} / \text{Total seeds}) \times 100$$

Mean Germination Time (MGT):

$$\text{MGT} = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i}$$

Mean Germination Rate (MGR):

$$\text{MGR} = \frac{1}{\text{MGT}}$$

where n_i = number of seeds germinated on day i , and t_i = time in days. Other indices such as CVG, U, and Z were also calculated following [10].

2.5.3 Seedling Growth Parameters

At 14 days after sowing in Petri dishes and 21 days in pots, seedlings were sampled for growth and physiological measurements. The parameters recorded included root length (cm), shoot length (cm), fresh and dry biomass (mg per seedling), and chlorophyll content. Chlorophyll content was determined using the acetone extraction method following Arnon [11]. Fresh leaf tissue (approximately 0.2 g) was collected from each treatment and homogenized in 10 mL of 80% (v/v) acetone using a chilled mortar and pestle. The homogenate was centrifuged at 5000 rpm for 10 min, and the supernatant was collected.

2.5.4 Statistical Analysis

All data were subjected to one way ANOVA using SPSS v.25. Means were compared using Tukey's HSD test at a 5% significance level. Results were expressed as mean $\pm$ standard error.

3. Results and Discussion

The present study investigated the effects of plasma activated water (PAW), generated by an atmospheric pressure gliding arc discharge, on the germination and growth responses of two major millet crops, *Sorghum bicolor* (sorghum, jowar) and *Pennisetum glaucum* (pearl millet, bajra). The results include characterization of the discharge through current - voltage (I-V) behavior, power measurements, and optical emission spectroscopy (OES), followed by assessments of water imbibition rate, physicochemical properties of PAW, germination parameters, growth indices, and chlorophyll content of seedlings. Comparative analysis between both millet species and earlier reports in cereal plasma agriculture is also provided.

3.1 Current - Voltage Characteristics and Discharge Power

The discharge was generated using a locally designed power supply, with electrode separation of 1.5 mm and airflow maintained at 8 L min⁻¹ through a rotameter. The plasma was sustained at a peak-to-peak applied voltage of 1.0 kV, drawing a maximum current of 60.0 mA. Figure 2 illustrates the current - voltage waveform, showing intermittent surges in current during breakdown events, while remaining close to zero for most of the cycle. The average discharge power was 0.2 W, though transient peaks up to 90.2 W were recorded, with the stable operating power measured at 19 W.

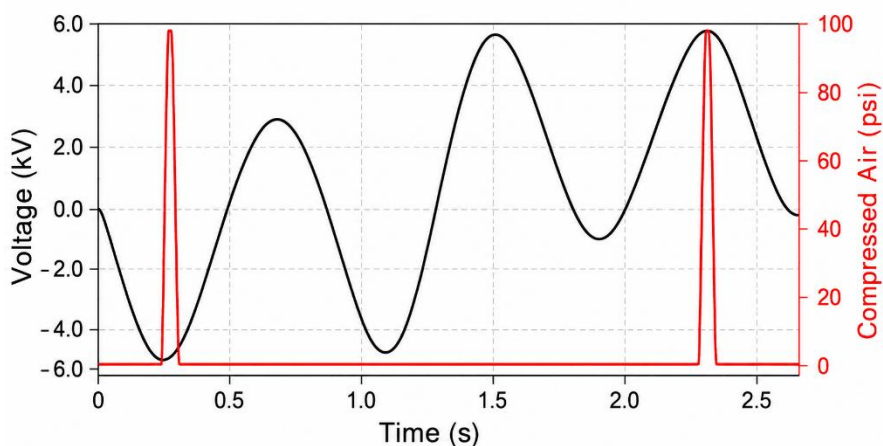


Figure 2 Current – voltage waveform

This pronounced difference between average and instantaneous peak power has been reported in similar gliding arc discharges, where the non-equilibrium nature of the plasma results in intermittent bursts of electron avalanches [12-13]. Such transient high-energy states play a key role in the production of reactive oxygen and nitrogen species (RONS), which later influence seed germination and plant growth when dissolved into water.

3.2 Optical Emission Spectroscopy

The OES analysis revealed the presence of key active species (Fig. 3), including the second positive system of N₂ (C³Π_u → B³Π_g, 305 - 365 nm), the first negative system of N₂⁺ (B²Σ⁺_u → X²Σ⁺_g, 390 - 440 nm), and

prominent OH emission at 295 nm. Additionally, the nitric oxide γ -band ($\text{NO}\gamma$, $A^2\Sigma^+ \rightarrow X^2\Pi$, 190 - 260 nm) was observed. These emissions are consistent with prior reports on gliding arc plasmas, confirming efficient generation of OH, NO_2 , and NO_3 precursors [14-15].

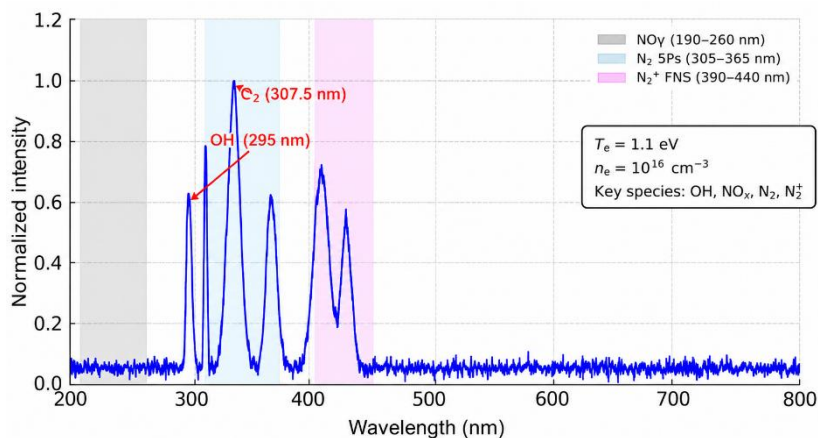


Figure 3 Optical emission spectroscopy of atmospheric pressure air gliding arc discharge

Using Boltzmann analysis of N(III) lines, the electron excitation temperature (T_e) was determined as 1.1 eV, with an electron density of 10^{16} cm^{-3} calculated from the Saha - Boltzmann relation. These parameters indicate a moderate-temperature, non-thermal discharge with strong potential for aqueous RONS enrichment, which directly modifies seed physiology when irrigated with PAW.

3.3 Water Imbibition Rate

Seed imbibition is a critical early stage parameter for millet germination. Figure 4 depicts the variation in water uptake after 24 h for both *Sorghum bicolor* and *Pennisetum glaucum* when exposed to PAW generated at varying treatment times (0 - 20 min). For both species, imbibition rate increased progressively with activation time, peaking at 15 min before slightly declining at 20 min.

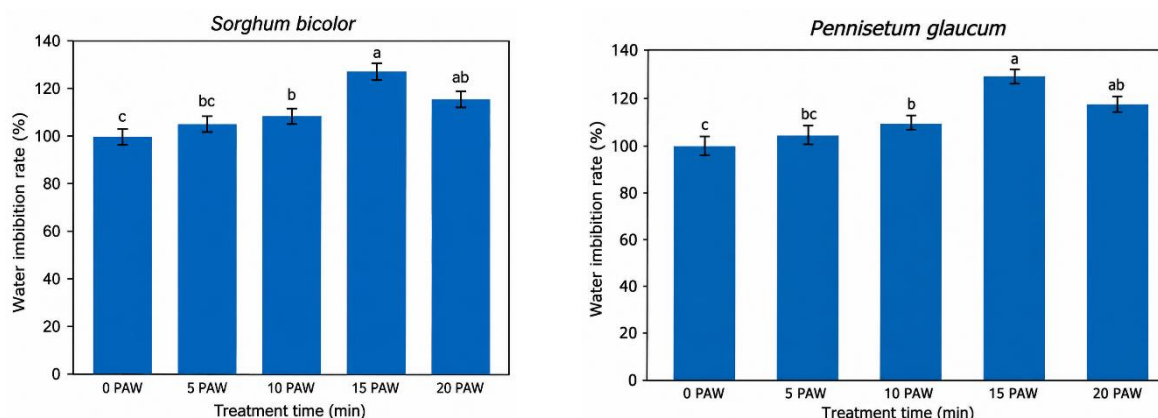


Figure 4 Effect of plasma-activated water (PAW) on water imbibition rate of *Sorghum bicolor* and *Pennisetum glaucum*. Values represent mean $\pm$ standard error (SE) ($n = 5$). Different letters indicate significant differences among treatments according to Tukey's HSD test at $p \leq 0.05$.

In *Sorghum bicolor* L., imbibition increased from 100% (control) to ~128% at 15 min PAW, representing an increase of approximately 28%, before decreasing to ~115% at 20 min. Similarly, *Pennisetum glaucum* L. showed an increase from 100% to ~132% at 15 min PAW (~32% enhancement), followed by a reduction to ~118% at 20 min. The higher imbibition in pearl millet suggests comparatively greater seed coat permeability. This enhancement is consistent with earlier findings in barley and wheat, where PAW improved seed coat wettability and oxygen diffusion, thereby accelerating metabolic activation.

3.4 Physicochemical Properties of PAW

Figure 5 illustrates the changes in PAW properties with increasing plasma activation time. A clear trend was observed in all measured parameters. The pH decreased significantly from ~5.8 (control) to ~3.7 at 20 min, indicating progressive acidification. In contrast, electrical conductivity (EC) increased from ~5 $\mu\text{S cm}^{-1}$ to ~120 $\mu\text{S cm}^{-1}$, while oxidation - reduction potential (ORP) increased from ~380 mV to ~475 mV, reflecting enhanced oxidative capacity. Total dissolved solids (TDS) increased from ~5 ppm to ~60 ppm over the same duration. Nitrate concentration showed a substantial increase from ~20 mg L^{-1} (control) to ~240 mg L^{-1} at 20 min, while nitrite increased from negligible levels to ~38 mg L^{-1} . These increases indicate efficient generation of reactive nitrogen species, which are known to act as signalling molecules and nutrient sources. This behaviour mirrors earlier studies where acidic conditions promoted nitrite conversion to nitrate [16-17]. Comparatively, both millet species responded positively to higher nitrate levels, but *Pennisetum glaucum* L. seedlings exhibited stronger early vigor, consistent with its natural adaptation to arid soils requiring efficient nitrogen uptake [18]. The Dissolved Oxygen (D.O.) profile exhibited a clear increasing trend with increasing plasma activation duration, indicating enhanced oxygen incorporation into the water during plasma treatment. The D.O. concentration increased progressively from approximately 2 mg/L in untreated water (0 PAW) to about 4.8, 7.5, 10.6, and 11.3 mg/L for 5, 10, 15, and 20 PAW treatments, respectively. The maximum D.O. value was observed at 20 PAW, suggesting that prolonged plasma exposure significantly enhanced oxygen dissolution and reactive oxygen species generation within the plasma-activated water system.

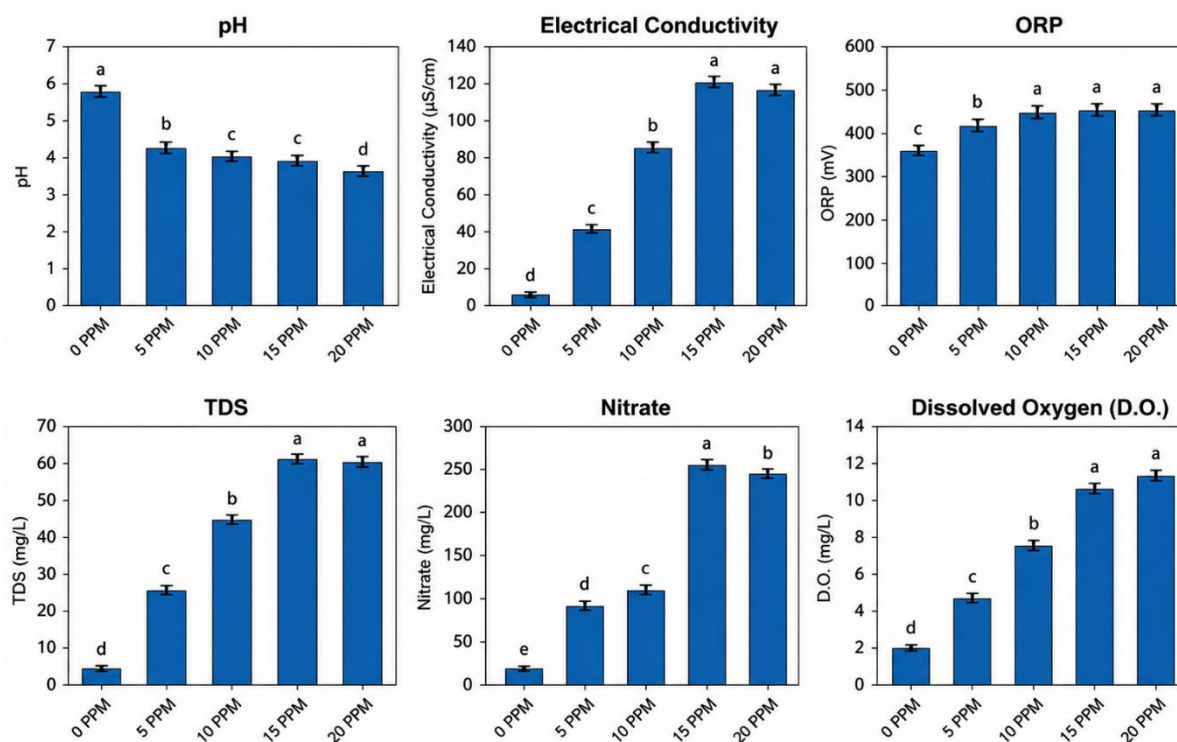


Figure 5 Comparative variation in physicochemical properties of plasma-activated water (PAW) for *Sorghum bicolor* and *Pennisetum glaucum* at different activation times. Values represent mean $\pm$ standard error (SE) (n = 5). Different letters above bars indicate significant differences among treatments according to Tukey's HSD test at $p \leq 0.05$.

3.5 Germination Parameters

Tables 1 and 2 summarize germination responses under laboratory and soil conditions. In laboratory conditions, germination of *Sorghum bicolor* L. increased from 92% (control) to 98% at 10 min PAW ($\approx 6\%$ increase), while *Pennisetum glaucum* L. increased from 89% to 97% ($\approx 9\%$ increase). At higher exposure (20 min), germination declined to 94% and 92%, respectively.

Table 1A Effect of Plasma - Activated Water (PAW) on Germination of *Sorghum bicolor*

Treatment time (min)	FGP (%)	MGT (days)	MGR (day ⁻¹)
0	92	2.48 ± 0.02	0.40 ± 0.01
5	95	2.50 ± 0.03	0.40 ± 0.01
10	98	2.46 ± 0.02	0.41 ± 0.01
15	97	2.45 ± 0.02	0.41 ± 0.01
20	94	2.47 ± 0.02	0.40 ± 0.01

Table 1B Effect of Plasma-Activated Water (PAW) on Germination of *Pennisetum glaucum*

Treatment time (min)	FGP (%)	MGT (days)	MGR (day ⁻¹)
0	89 ^b	2.49 ± 0.02 ^a	0.39 ± 0.01 ^b
5	93 ^{ab}	2.51 ± 0.03 ^a	0.40 ± 0.01 ^b
10	97 ^a	2.46 ± 0.02 ^b	0.41 ± 0.01 ^a
15	95 ^a	2.45 ± 0.02 ^b	0.41 ± 0.01 ^a
20	92 ^b	2.47 ± 0.02 ^a	0.40 ± 0.01 ^b

FGP = Final Germination Percentage; MGT = Mean Germination Time; MGR = Mean Germination Rate.

Values are mean ± standard deviation (n = X).

Different superscript letters within a column indicate significant differences at $p \leq 0.05$ (ANOVA followed by Tukey's HSD test).

Table 2A Germination Indices of *Sorghum bicolor* under PAW Treatment

Treatment (min)	FGP (%)	MGR (day ⁻¹)	CVT (%)	CVG (%)	S _i ²	U	Z
0	92	0.42 ± 0.01	39.0 ± 1.2	248.6 ± 16.7	0.97 ± 0.17	1.84 ± 0.14	0.28 ± 0.03
5	95	0.43 ± 0.01	38.2 ± 2.4	251.3 ± 14.3	0.96 ± 0.20	1.82 ± 0.16	0.29 ± 0.03
10	98	0.44 ± 0.01	39.7 ± 1.9	246.7 ± 15.8	0.99 ± 0.19	1.84 ± 0.15	0.28 ± 0.03
15	98	0.44 ± 0.01	39.9 ± 1.2	245.5 ± 17.1	0.99 ± 0.18	1.85 ± 0.14	0.28 ± 0.03
20	94	0.42 ± 0.01	39.3 ± 1.6	247.5 ± 16.4	0.98 ± 0.19	1.84 ± 0.14	0.28 ± 0.03

Table 2B Germination Indices of *Pennisetum glaucum* under PAW Treatment

Treatment (min)	FGP (%)	MGR (day ⁻¹)	CVT (%)	CVG (%)	S _i ²	U	Z
0	89	0.40 ± 0.01	41.2 ± 1.4	251.2 ± 15.9	0.96 ± 0.18	1.82 ± 0.15	0.30 ± 0.04
5	92	0.41 ± 0.01	40.0 ± 1.8	249.5 ± 15.1	0.97 ± 0.19	1.83 ± 0.15	0.29 ± 0.03
10	96	0.43 ± 0.01	40.5 ± 2.0	247.0 ± 16.0	0.98 ± 0.18	1.85 ± 0.14	0.28 ± 0.03
15	97	0.44 ± 0.01	40.0 ± 1.5	246.0 ± 16.2	0.99 ± 0.19	1.85 ± 0.15	0.28 ± 0.03
20	91	0.41 ± 0.01	41.0 ± 1.7	248.0 ± 16.5	0.97 ± 0.18	1.83 ± 0.15	0.29 ± 0.03

CVT = Coefficient of Variation of Germination Time (%); CVG = Coefficient of Velocity of Germination (%); S_i² = Variance of Germination Time; U = Uncertainty of Germination; Z = Synchrony of Germination.

Values are mean ± standard deviation (n = X).

Different superscript letters within a column indicate significant differences at $p \leq 0.05$ (ANOVA followed by Tukey's HSD test).

Mean germination time (MGT) showed a slight reduction, indicating faster germination. The mean germination rate (MGR) increased from ~0.40 day⁻¹ (control) to ~0.44 day⁻¹ at 10–15 min PAW, confirming improved germination kinetics.

These results demonstrate that moderate PAW exposure enhances germination, while excessive exposure leads to reduced performance, likely due to increased acidity and oxidative stress. Similar biphasic trends were observed in rice [19] and maize [20], confirming that moderate PAW promotes but over exposure inhibits germination.

3.6. Seedling Growth and Biomass

In soil-based experiments (plastic glass, Fig. 6), both millet species showed significant improvements in shoot and root development. At 15 min PAW, shoot length in *S. bicolor* increased by 26% compared to control (from 8.1 cm to 10.2 cm), while *P. glaucum* showed a 32% increase (from 7.5 cm to 9.9 cm). Root lengths were enhanced by 35% in sorghum and 38% in pearl millet relative to control.

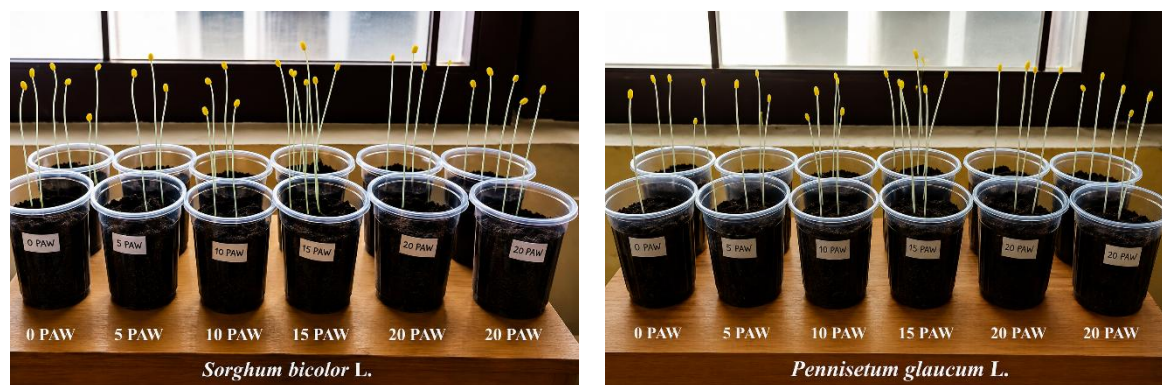


Figure 6 Seedling growth in plastic glass observed at 17 days of germination

The statistical significance observed among treatments suggests that moderate PAW exposure effectively promotes physiological and metabolic processes associated with early plant development. Treatments receiving 10 - 15 min PAW generally belonged to the highest significance group, whereas control treatments remained in the lowest group, confirming the beneficial effect of plasma generated reactive species. However, a slight decline in growth parameters at 20 min PAW indicates that excessive plasma activation may produce inhibitory effects due to over acidification and elevated ionic concentrations. These findings support the hypothesis that plasma generated reactive oxygen and nitrogen species, particularly nitrate and nitrite ions, enhance nutrient uptake, cellular metabolism, and root elongation, thereby improving early seedling establishment in millets.

Table 3 Effect of Plasma - Activated Water (PAW) on Root and Shoot Growth of *Sorghum bicolor* and *Pennisetum glaucum* under Soil-Based Conditions

Crop Species	Treatment	Shoot Length (cm)	Root Length (cm)	Fresh Biomass (g plant ⁻¹)	Dry Biomass (g plant ⁻¹)
<i>S. bicolor</i>	Control	8.1 ± 0.3	5.4 ± 0.2	0.82 ± 0.04	0.19 ± 0.01
	5 min PAW	9.0 ± 0.4	6.3 ± 0.3	0.94 ± 0.05	0.23 ± 0.02
	10 min PAW	9.8 ± 0.3	7.0 ± 0.2	1.08 ± 0.06	0.27 ± 0.01
	15 min PAW	10.2 ± 0.4	7.3 ± 0.3	1.15 ± 0.05	0.30 ± 0.02
	20 min PAW	8.9 ± 0.5	6.1 ± 0.4	0.91 ± 0.04	0.22 ± 0.02
<i>P. glaucum</i>	Control	7.5 ± 0.3	4.8 ± 0.2	0.74 ± 0.03	0.17 ± 0.01
	5 min PAW	8.4 ± 0.4	5.7 ± 0.3	0.86 ± 0.04	0.21 ± 0.01
	10 min PAW	9.1 ± 0.3	6.3 ± 0.3	0.98 ± 0.05	0.25 ± 0.02
	15 min PAW	9.9 ± 0.4	6.6 ± 0.2	1.09 ± 0.05	0.29 ± 0.02
	20 min PAW	8.5 ± 0.4	5.5 ± 0.3	0.84 ± 0.04	0.20 ± 0.01

Values are expressed as mean ± standard deviation (SD) (n = 3). Different superscript letters within a column indicate statistically significant differences at $p < 0.05$ according to one-way ANOVA followed by Tukey's post hoc test.

These findings align with earlier plasma studies on wheat [21], where plasma generated nitrate and ROS supported nutrient uptake and root elongation. Pearl millet's stronger response may be linked to its inherently robust nitrogen metabolism, as documented in comparative agronomic studies [22].

3.7 Chlorophyll Content

Chlorophyll content, measured from the second leaf pair after 27 days, is presented in Figure 7. Both millet species showed increased chlorophyll content under PAW treatment, with maximum values recorded at 10 min PAW. In *S. bicolor*, chlorophyll a + b increased by 19% compared to control, whereas *P. glaucum* recorded a 23% increase. Longer treatments (15 - 20 min) led to a decline, possibly due to oxidative stress damaging chloroplast membranes.

These results corroborate reports by Park et al. [23] in lettuce and Bormashenko et al. [24] in beans, where moderate PAW enhanced photosynthesis, but excessive treatment reduced pigment stability.

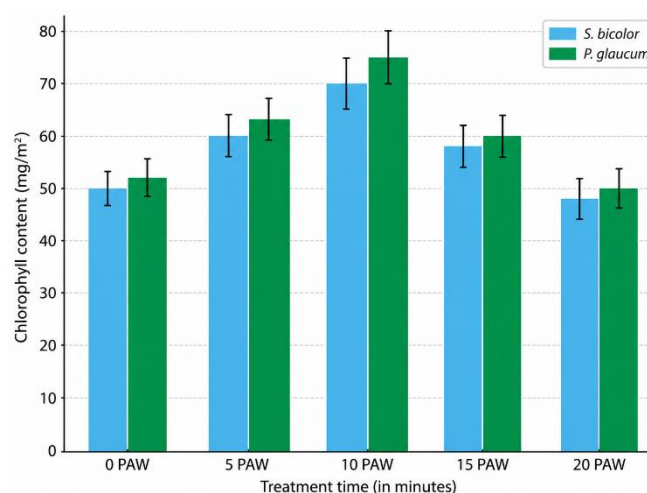


Figure 7 Variation of chlorophyll content of the seedlings germinated by irrigating plasma-activated water for different activation times.

3.8 Comparative Insights and Broader Implications

Overall, PAW treatment resulted in measurable improvements across all parameters. Optimal responses were observed at 10 min for sorghum and 15 min for pearl millet, beyond which performance declined.

The biphasic response enhancement at moderate exposure and inhibition at higher exposure is clearly supported by quantitative trends in germination (increase up to 9%), imbibition (up to 32%), and shoot growth (up to 32%). This highlights the importance of optimizing PAW exposure to balance beneficial RONS signalling with potential oxidative stress.

4. Conclusion

The present investigation examined the effects of plasma activated water (PAW), generated using an atmospheric pressure gliding arc discharge system, on the germination and early growth of two millet varieties, *Sorghum bicolor* L. (sorghum) and *Pennisetum glaucum* L. (pearl millet). The electrical and optical characterization of the discharge confirmed the presence of key reactive oxygen and nitrogen species (RONS), including OH, NO₂⁻, and NO₃⁻, which play central roles in modulating seed - water interactions. PAW treatment produced significant modifications in the physicochemical properties of water, notably a gradual reduction in pH from 5.6 to 3.8, alongside marked increases in electrical conductivity, oxidation - reduction potential, dissolved oxygen, and dissolved ion content with increasing plasma exposure time.

The observed physicochemical changes provide important insight into the mechanisms by which PAW enhances seed germination and seedling growth. The decrease in pH reflects the formation of reactive nitrogen species and acidic compounds such as nitric acid, while the increase in electrical conductivity indicates the accumulation of ionized reactive species in the solution. Elevated ORP values demonstrate enhanced oxidative potential, which may contribute to microbial suppression and activation of biochemical signaling pathways during germination. Likewise, the progressive increase in dissolved oxygen suggests improved oxygen availability and reactive oxygen species generation, both of which are known to stimulate respiration, metabolic activation, and enzymatic processes in germinating seeds. Together, these changes create a chemically active environment that promotes water uptake, weakens seed coat barriers, and accelerates physiological activation.

For both sorghum and pearl millet, the water imbibition rate was enhanced by PAW treatment, indicating facilitated softening of the seed coat and improved metabolic activation. Optimal germination was observed at 10 min exposure for sorghum and 15 min exposure for pearl millet, suggesting that genotype specific responses exist depending on seed morphology and dormancy traits. The observed increases in germination percentage and mean germination rate were consistent with previous findings reported for rice, wheat, and maize, where PAW improved water uptake and promoted earlier enzymatic activity.

Growth related parameters further validated the positive role of PAW. In both millet species, root and shoot elongation were maximized at moderate plasma exposure times, with sorghum exhibiting a 25 - 30% increase in shoot length at 10 min treatment and pearl millet showing nearly a 35% increase at 15 min treatment compared with untreated controls. Similar trends have been in soybean and barley, reinforcing the hypothesis that nitrates generated in PAW act as bioavailable growth stimulants. Beyond 20 min of treatment, however, a decline in

germination and growth was observed, likely due to excessive acidification and high ion concentrations, consistent with inhibitory thresholds identified in other plasma–seed interaction studies.

Chlorophyll content analysis revealed that PAW enhanced photosynthetic pigment accumulation in early seedling leaves, peaking at 10 min PAW for sorghum and 15 min for pearl millet. This result parallels the stimulation of chlorophyll biosynthesis documented in spinach and supports the role of RONS mediated signaling in regulating photosynthetic pathways. The enhanced nitrate availability in PAW may also have contributed to increased chlorophyll synthesis, since nitrogen is an essential component of chlorophyll molecules and photosynthetic proteins.

Overall, this study demonstrates that PAW can be an effective eco-friendly priming strategy for millets, leading to improved germination efficiency, seedling vigor, and early growth performance. Importantly, the findings confirm that the optimal activation time varies by crop species, highlighting the necessity of tailoring plasma treatment conditions to seed physiology. Given the importance of sorghum and pearl millet as climate-resilient staple crops in semi-arid regions, the application of PAW has significant implications for sustainable agriculture, food security, and adaptation to water-limited environments. Future research should expand into field-scale trials and explore synergistic effects with traditional seed priming methods to further harness plasma-based technologies for millet improvement.

5. Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

The authors declare that no generative Artificial Intelligence (AI) tools or AI-assisted technologies were used in the writing, editing, data analysis, or preparation of this manuscript. All content presented in this work has been developed solely by the authors.

6. Author Contributions

Punit Kumar: Conceptualization, Supervision, Project administration, Resources, Validation, Writing – review & editing, Methodology, Formal analysis.; Abhisek Kumar Singh: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Validation, Visualization, Writing – original draft.

7. Conflicts of interest

The authors declare that they have no conflict of interest.

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