**To appear in:**

# Journal of Theoretical and Applied Physics

**Online ISSN: 2251-7235** **Print ISSN: 2251-7227**



Received: 08 November 2025
Revised: 25 December 2025
Accepted: 09 April 2026

DOI: https://doi.org/10.57647/jtap.2026.2004.08

Research Article

# Effects of Direct and Indirect Cold Plasma Treatments on Barley (*Hordeum vulgare* L.) Seed Germination and Seedling Growth

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## Abstract

This study presents a comparative evaluation of direct and indirect cold plasma treatments on seed germination and seedling growth of barley (Hordeum vulgare L. cv. Sahra). Direct treatments was performed using low-pressure nitrogen glow discharge under ion-dominant (P1) and electron-dominant (P2) conditions, while indirect treatments employed plasma-activated water (PAW) generated via atmospheric-pressure dielectric barrier discharge (DBD) in deionized (PAW1) and tap water (PAW2). Germination rate, seedling vigor index, and early growth parameters were assessed under controlled conditions. The ion-dominant plasma treatment (P1) showed the highest germination rate (93.3%) and seedling vigor index (7082.0), outperforming PAW1 (79.7%), PAW2 (73.3%), and control (72.0%). In contrast, electron-dominant treatment (P2) reduced germination (60.3%), demonstrating the crucial role of plasma species composition in biological responses. The enhanced efficacy of PAW was associated with its physicochemical properties, particularly a reduced pH (3.42) and increased conductivity (174 µS/cm for PAW1, 1281 µS/cm for PAW2), which may facilitate the stabilization of reactive nitrogen species and improve seed coat permeability. Optical emission spectroscopy and water contact angle measurements supported plasma-induced surface hydrophilization and the presence of excited nitrogen species under ion-dominant conditions. These findings establish ion-dominant nitrogen cold plasma as an effective, chemical-free seed priming strategy and emphasize the importance of plasma regime control in optimizing sustainable agricultural applications.

**Keywords**: Barley, Cold Plasma, Glow Discharge, Plasma-Activated Water, Seed Priming, Sustainable Agriculture

## 1. Introduction

Barley (*Hordeum vulgare* L.) is a significant cereal crop worldwide, ranking as the fourth most important after maize, wheat, and rice. As a valuable grain in agriculture and industry, it is utilized for animal feed, human food, and as a key ingredient in the malting industry. According to the Food and Agriculture Organization (FAO), global barley production reached approximately 148 million tons in 2024, with Iran contributing roughly 3.6 million tons, representing nearly 2.5% of the total yield [1]. Despite its agricultural value, barley cultivation faces challenges during early growth, particularly germination and seedling stages [2,3]. These challenges have led researchers to investigate new methods, such as plasma treatments, to address problems related to germination efficiency and seedling establishment [4,5].

Germination is a crucial phase in the lifecycle of a plant, during which seeds initiate metabolic processes essential for early growth. This stage is strongly regulated by multiple physiological and environmental factors, including water availability, temperature, and oxygen concentrations [6,7]. In barley, germination is further hindered by its thick outer husk, which constitutes roughly 10% of seed mass and restricts water uptake and gas exchange [8]. Traditional methods, for instance, mechanical scraping, hydropriming, cold stratification, and chemical soaking, have been widely used to break dormancy and enhance seed germination. Nevertheless, these conventional approaches often face limitations, for example, extended treatment times, possible toxicity, and microbial contamination [9].

In recent years, non-thermal plasma (NTP) technology has emerged as a potential substitute for seed priming by generating reactive oxygen and nitrogen species (RONS), UV photons, and charged particles [10-14]. These components physically modify the seed coat through micro-cracking and surface etching, thereby improving water absorption [11]. In contrast, reactive nitrogen species (RNS), including nitric oxide (NO) and nitrogen dioxide ($NO_2$), activate genes and enzymes, such as amylase and protease, critical for breaking seed dormancy and promoting radicle emergence [8,11]. The effect of NTP treatments depends on several parameters, particularly the type of gas, discharge power, and treatment duration, as well as seed characteristics, like husk thickness and moisture content [15-17].

Two principal approaches to plasma application in agriculture have been developed: (i) direct plasma exposure, where seeds are treated within the plasma discharge zone, and (ii) indirect application via plasma-activated water (PAW). In this case, plasma-generated reactive oxygen and nitrogen species, particularly $NO_3^-$ and $H_2O_2$, are transferred into water and used for irrigation or seed soaking [18]. PAW, enriched with long-lived species, can enhance seedling growth, disinfect seeds, and act as a nitrogen fertilizer [18,19]. Nitrate ions in PAW act similarly to potassium nitrate, a well-known germination enhancer under stress conditions, highlighting the role of $NO_3^-$ as a bioactive agent [19]. However, its complex mechanisms remain insufficiently understood and require further investigation.

Numerous studies have demonstrated the role of cold plasma in enhancing seed germination and seedling performance of barley. In 2015, Braşoveanu et al. used glow discharge plasma on barley seeds to reduce the number of seed-borne fungal contamination, enhancing germination and growth parameters [20]. Conversely, Dubinov et al. (2010) reported species-dependent responses, with barley showing minimal improvement under pulsed air plasma [21]. More recent studies by Sivachandiran and Khacef (2017) and Peťková et al. (2016) confirmed that plasma-induced surface changes improve water uptake and germination uniformity in barley [22, 23]. A comprehensive study by Benabderrahim et al. (2024) explored three distinct cold plasma treatment modalities on barley seeds: direct treatment of dry seeds (DDS), direct treatment of water-soaked seeds (DWS), and indirect treatment using plasma-activated water (IPAW) [13]. Their findings revealed that DDS significantly increased germination to 78% compared to 42% for controls, while DWS inhibited germination due to oxygen deficiency and oxidative stress. Seleiman et al. (2024) addressed PAW's low pH limitation by developing magnesium-neutralized PAW (Mg-PAW), which outperformed standard PAW in barley germination and seedling growth [24].

Although significant progress has been made, comparative studies on direct low-pressure glow discharge and PAW generated via atmospheric-pressure dielectric barrier discharge (DBD) in barley remain limited. The interplay between the parameters of plasma treatment and the quality of water (such as deionized versus tap water) on the properties of PAW and its biological responses is not fully elucidated. Considering the morphological and physiological characteristics of barley seeds, additional research is needed to optimize plasma treatment conditions for improved germination and early seedling growth.

In this study, the influences of two cold plasmas produced by a low-pressure nitrogen glow discharge generator, as well as the plasma-activated water, on the germination and growth characteristics of barley seeds have been systematically investigated. Both deionized water and tap water were activated to produce PAW and applied under controlled conditions. Various physiological parameters, including the germination rate, root and shoot length, and the seedling vigor index, were evaluated across different treatment groups. To determine the significance of differences among treatment groups, statistical analyses such as one-way ANOVA and subsequent LSD tests were performed. Furthermore, the physicochemical characteristics of PAW, e.g., pH and electrical conductivity, with a particular emphasis on the role of reactive species, were examined. This study uniquely investigates the differential effects of ion- versus electron-dominated plasma treatment and the influence of water quality on PAW efficacy, providing new insights into optimizing plasma-based seed priming for barley.

## 2. Materials and Experimental Methods

### 2.1. Seed Material and Preparation

Barley seeds (*Hordeum vulgare* L., cv. Sahra) were obtained from the Golestan Agricultural and Natural Resources Research Center, Iran. Seeds were manually cleaned to remove broken, shriveled, or visibly damaged samples and were sieved through a 2.5 mm mesh for uniformity. Samples were maintained in airtight containers at room temperature (22–25 °C) and processed within four weeks to avoid potential aging effects.

### 2.2. Direct Plasma Treatment via Glow Discharge

The fundamental approach to generating cold plasma involves ionization of a gas medium through the application of an electric discharge between the electrodes of a plasma-generating device. Figure 1 illustrates a schematic representation of the experimental setup. In this experiment, cold plasma was generated using a custom-designed glow discharge system, offering a cost-effective and straightforward configuration. It included a cylindrical metallic chamber equipped with a radio-frequency (RF) power supply operating in the range of 50-300 W, a Pirani gauge for monitoring pressure, and a rotary vacuum pump attached to the bottom of the chamber. High-purity nitrogen (99.99%) was flowed at a rate of 2 L/min into the chamber through the inlet located at the top. The working pressure was regulated to approximately $1\times10^{-2}$ Torr before discharge. Two parallel metallic electrodes, 10 cm in diameter and 6 cm apart, act like two charged capacitors and serve as the discharge plates. Barley seeds were placed between the electrodes and exposed to nitrogen plasma at 300 W power and 500 V discharge voltage for 60 seconds. The tests are performed within the pressure ranges of up to $6\times10^{-1}$ Torr.

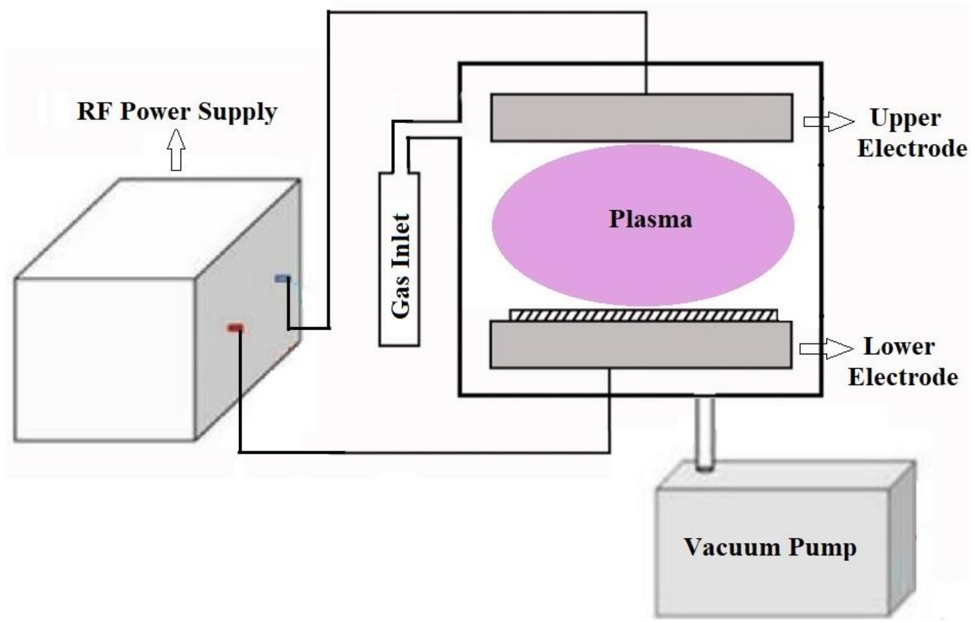


Figure 1: Schematic of the low-pressure glow discharge plasma system used for direct barley seed treatment, operating at $6\times10^{-1}$ Torr with 300 W power and nitrogen gas flow of 2 L/min.

To distinguish between the effects of plasma species, seeds were placed either on the cathode (electron-dominant exposure) or anode (ion-dominant exposure), as illustrated in Figure 2. This arrangement allowed for selective interaction with accelerated nitrogen ions (Figure 2a) or electrons (Figure 2b).

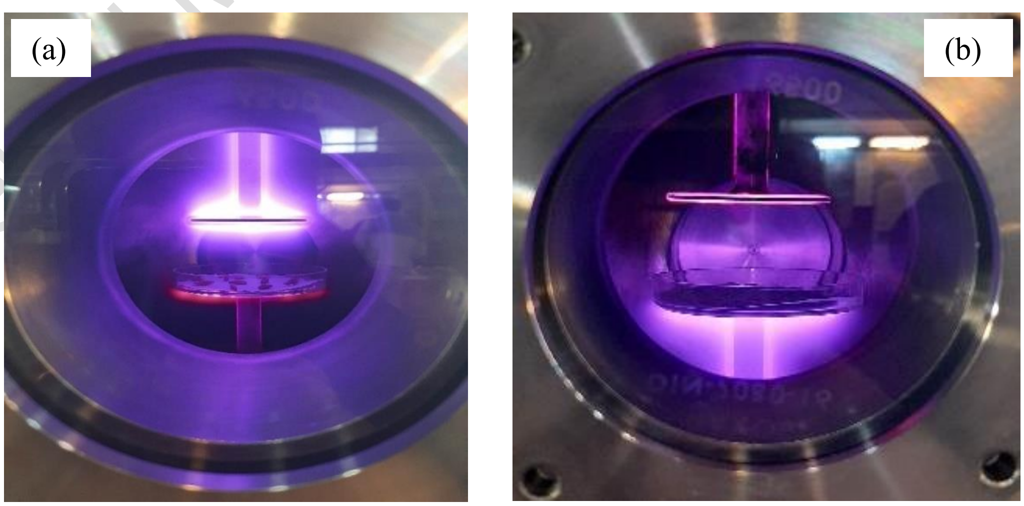

Figure 2: Barley seeds treated with (a) ion-dominated plasma exposure, (b) electron-dominated plasma exposure using low-pressure nitrogen glow discharge ($6\times10^{-1}$ Torr, 300 W RF, 60 s treatment time).

### 2.3. Plasma-Activated Water Preparation via DBD

Plasma-activated water was produced using a DBD system operated under atmospheric pressure with high-purity nitrogen gas (99.99%). The DBD setup comprised two parallel stainless-steel electrodes separated by a 3-mm thick quartz plate acting as a dielectric barrier. The plasma discharge was powered by a sinusoidal high-voltage power supply operating at 15 kV peak-to-peak voltage, 15 kHz frequency, and an average input power of 60 W. To investigate the effects of PAW on seed germination, two types of water were used as base media: deionized water and tap water. For each treatment, 200 mL of water was placed in a sterilized glass beaker. The electrodes were immersed in the liquid, and the plasma discharge was initiated and sustained for a treatment duration of 10 minutes. During activation, nitrogen gas was bubbled through the water at a constant flow rate of 0.5 L/min to enhance the dissolution of plasma-generated reactive species (see Figure 3).

Subsequently, the physicochemical characteristics of the prepared PAW, including acidity (pH), electrical conductivity (EC), and total dissolved solids (TDS), were measured in triplicate immediately after treatment. pH was measured with a calibrated Inolab pH 7110 meter (accuracy: ±0.001) using standard buffer solutions of pH 4.00 and 7.00. Electrical conductivity (EC) and total dissolved solids (TDS) were evaluated using an AZ8603 multiparameter water quality meter (accuracy: ±0.01 μS/cm for EC and ±0.01 mg/L for TDS; AZ Instrument Corp., Taiwan).

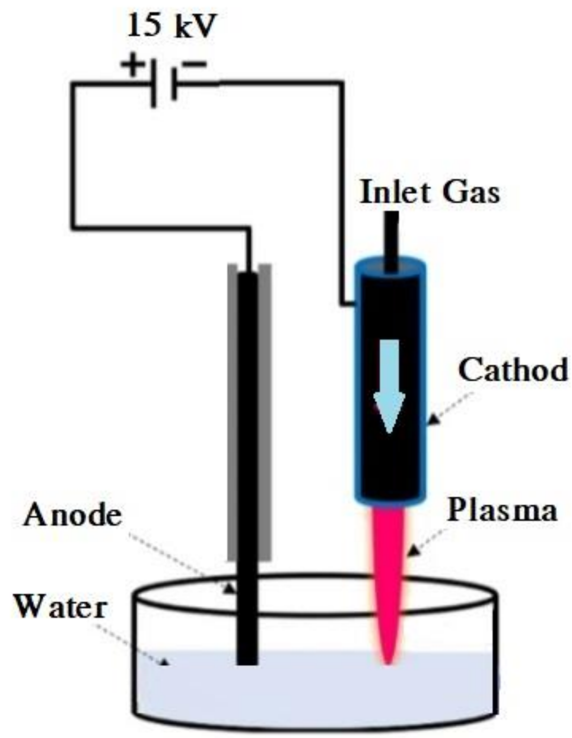


Figure 3: Schematic of the plasma-activated water preparation setup using a dielectric barrier discharge (DBD) reactor

### 2.4. Seed Germination and Growth Experiment

Treated and untreated barley (Hordeum vulgare L. cv. Sahra) seeds were placed in sterile 9-cm Petri dishes lined with filter paper (Figure 4). Untreated seeds irrigated with untreated deionized water served as the control group. Each Petri dish received 3 mL of either plasma-activated deionized water (PAW1), plasma-activated tap water (PAW2), or untreated deionized water following direct plasma treatment (P1: ion-dominant plasma; P2: electron-dominant plasma). After 24 hours, dishes were transferred to a germination chamber maintained at 20 °C and 50% humidity with 16/8 h light/dark cycle. Germination was monitored daily for 14 days, and the germination rate was recorded, according to the International Rules for Seed Testing (ISTA) [25].

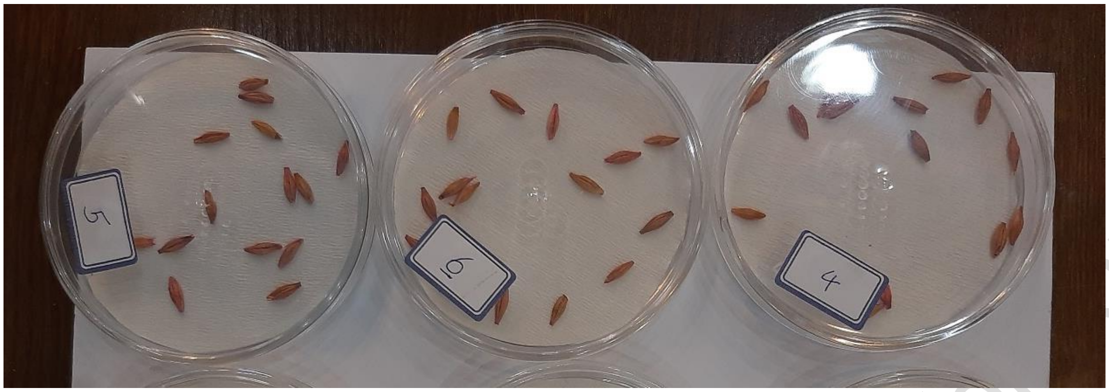


Figure 4: A representative Petri dish containing barley seeds placed on filter paper for seed germination

In cereal crops like barley, germination is defined as the emergence of the radicle to a length of at least 2 mm, per ISTA criteria [25]. Based on the standard germination protocol, germination rate was calculated on day 7 using the formula from Maguire (1962) [26]:

Germination rate (%)= (Number of germinated seeds/ Total number of seeds) ×100.

For each treatment, three biological replicates (Petri dishes) containing 15 seeds (n = 45 seeds per treatment) were used. On days 9 and 14, root and shoot lengths were measured using a millimeter-scale ruler. To evaluate biomass accumulation, fresh and dry weights of whole seedlings were measured on day 14. Fresh weight was determined immediately using an analytical balance (accuracy ±0.001 g). Samples were then oven-dried at 70 °C for 48 h until constant weight to obtain dry weight (mg/seedling). The Seedling Vigor Index (VI), which indicates the overall growth potential of the seeds, was calculated according to Abdul-Baki and Anderson (1973) [27]:

Seedling Vigor Index = (Root length + Shoot length) × germination percentage

### 2.5. Statistical Analysis

All data analyses were conducted using SPSS version 26.0 (SPSS Inc., Chicago, IL, USA). The normality of the data was confirmed with the Shapiro–Wilk test. Identification of differences between the treatments was performed using a one-way analysis of variance (ANOVA). In cases where significant differences were found ($P \leq 0.05$), Fisher's Least Significant Difference (LSD) post hoc test was employed to compare treatment means in pairs. All data are presented as mean ± standard error of the mean (SEM), and error bars are explicitly shown in Figures.

### 2.6. Optical Emission Spectroscopy

Optical emission spectroscopy (OES) technique was employed to identify the dominant excited species generated in the nitrogen glow discharge plasma. Emission spectra were recorded in the wavelength range of 300-900 nm using a fiber-optic spectrometer positioned perpendicular to the plasma column at a fixed distance from the discharge region. The spectrometer integration time and acquisition settings were kept constant throughout the measurements. Measurements were performed under the standard operating condition (300 W RF power, $6\times10^{-1}$ Torr, 2 L/min $N_2$ flow) applied to ion-dominant seed exposure.

### 2.7. Seed Water Contact Angle

Apparent water contact angle (WCA) measurements were performed to quantify plasma-induced changes in the surface wettability of barley seeds. Static sessile-drop experiments were conducted at room temperature under ambient conditions, with the apparent contact angle determined by axisymmetric drop profile fitting. A fixed volume of 1 µL deionized water droplet was gently deposited onto the seed surface using a precision syringe. Contact angle images were captured immediately after droplet deposition using a CCD camera to minimize evaporation-related artifacts, and apparent contact angles were quantified via digital image analysis software (ImageJ). WCA measurements were restricted to untreated seeds and seeds directly treated under ion-dominated nitrogen plasma conditions for exposure times of 1, 3, and 5 min, selected based on prior optimization experiments demonstrating stronger surface activation under ion-dominated regimes. For each treatment, measurements were performed on at least three independent seeds ($n \geq 3$), and results are reported as mean ± SEM.

## 3. Results and Discussions

### 3.1. Seed Germination Response to Plasma Treatments

The effects of two plasma-based treatments on the seed germination of barley were evaluated by comparing direct exposure to nitrogen glow-discharge plasma (P1: ion-dominant and P2: electron-dominant) with indirect exposure via plasma-activated water. This included PAW1 for deionized water and PAW2 for tap water. As shown in Figure 5, the P1 treatment achieved the highest germination rate at 93.3 ± 0.3%. Germination rates were recorded as 79.7 ± 0.9% for PAW1, 73.3 ± 1.8% for PAW2, and 72.0 ± 1.5% for the untreated control. Conversely, P2 showed the lowest germination rate at 60.3 ± 1.2%.

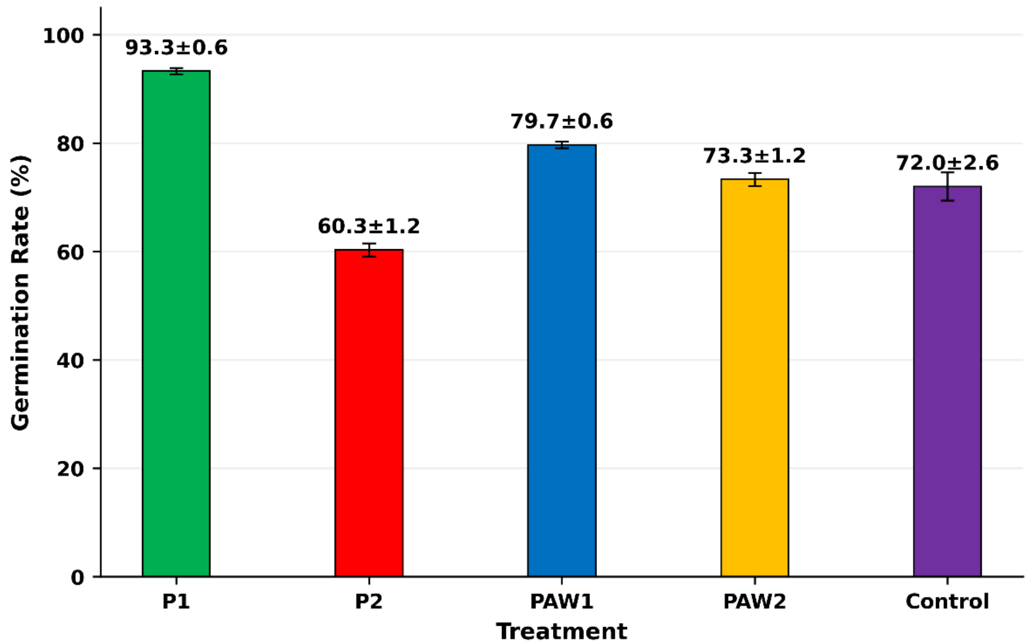


Figure 5: Germination rate (%) of barley seeds on day 7 under direct plasma (P1, P2), plasma-activated water (PAW1, PAW2), and control treatments. Error bars represent the standard error of the mean (SEM).

These results are summarized in Table 1, which presents the germination rates for all treatment groups after 7 days. The one-way ANOVA statistical analysis indicated remarkably significant differences among the treatments ($P < 0.0001$), confirming the positive impact of plasma-based approaches on seed germination.

Table 1: Germination rate of barley seeds under different treatments expressed as mean ± SEM

| **Treatment** | **P1 (ion-dominant)** | **P2 (electron-dominant)** | **PAW1 (deionized water)** | **PAW2 (tap water)** | **Control** |
|---|---|---|---|---|---|
| **Germination rate(%)** | 93.3 ± 0.6 | 60.3 ± 1.2 | 79.7 ± 0.6 | 73.3 ± 1.2 | 72.0 ± 2.6 |

Additional post hoc LSD analysis validated that P1 outperformed all other treatments ($P < 0.05$), emphasizing the dominant effect of direct nitrogen-ion exposure in breaking seed dormancy and enhancing germination [4, 28- 30]. This visual comparison is presented in Figure 6, showing barley seed germination on day 7 for the P1-treated group and the untreated control. Enhanced radicle emergence and germination density are visible in the P1-treated sample. In contrast, electron-dominated plasma exposure (P2) significantly reduced germination, suggesting that the type of reactive species plays a critical role in modulating biological responses.

The enhanced germination observed in P1 may be associated with a substantial flux of nitrogen ions, on the order of $10^{15}$ $cm^{-2}s^{-1}$, as reported for comparable nitrogen glow discharge systems [31], together with an electron temperature typically ranging from 2 to 3 eV. These can lead to the formation of micro-cracking and the introduction of polar groups such as carboxyl and hydroxyl [11, 31]. These modifications can enhance the permeability of the seed coat, facilitating water absorption and the activation of metabolic processes.


Figure 6: Visual comparison of barley seed germination on day 7. (left) ion-dominated plasma (P1) treatment, (right) untreated control.

The dominant germination performance observed with PAW1 (plasma-activated deionized water) compared to PAW2 (plasma-activated tap water) can primarily be due to the distinct in physicochemical characteristics, as presented in Table 2. PAW1 demonstrated a significantly lower pH (3.42) and moderate electrical conductivity (174 µS/cm), conditions that are conducive to the generation and stabilization of RNS and reactive oxygen species (ROS), such as NO, $NO_2$, and $H_2O_2$. These compounds facilitate seed germination by influencing nitrate signaling pathways and enhancing enzymatic activity [32-34]. In contrast, PAW2 and untreated tap water displayed substantially elevated conductivity levels (1281 and 875 µS/cm, respectively), which may result in osmotic stress on soaking seeds, thereby inhibiting water uptake and postponing metabolic activation [24, 30, 33, 35].

These findings align with recent studies reporting that the bioactivity of PAW is highly dependent on both the reactive species content and baseline water quality.

For instance, Adhikari et al. [36] demonstrated that the composition of PAW significantly influences seed germination and early growth, thereby supporting the notion that deionized water offers a more controlled and chemically responsive medium for plasma-induced modifications. Similarly, Benabderrahim et al. [13] pointed out that the physicochemical properties of PAW, especially its pH and conductivity, have a direct impact on the germination rates of various crops, including barley.

Consequently, optimizing the composition of PAW, with a focus on its pH and conductivity, is essential for enhancing its role as a seed priming agent in plasma-assisted agriculture. This perspective is reinforced by findings from Ferreyra et al. [33], who emphasize that adjusting the physicochemical properties of PAW can enhance its effectiveness for various plant types, thus opening up avenues for more efficient agricultural practices using plasma technology.

Table 2: Physicochemical properties of plasma-activated water (mean ± SEM)

| **Parameter** | **Plasma-activated deionized water (PAW1)** | **Plasma-activated tap water (PAW2)** | **tap water** | **deionized water** |
|---|---|---|---|---|
| **pH** | 3.42±0.03 | 7.4±0.05 | 7.8±0.1 | 7.06±0.05 |
| **EC ($\mu S/cm$)** | 174±4.2 | 1281±28 | 875±14 | 2±0.5 |
| **TDS (mg/L)** | 69±2.1 | 620±12 | 648±20 | 0.7±0.2 |

### 3.2. Seedling Growth Performance

Table 3 shows the lengths of roots and shoots on days 9 and 14, as well as seedling fresh and dry biomass, for barley under different plasma treatments. As indicated in Table 3, direct nitrogen plasma (P1) and plasma-activated tap water (PAW2) demonstrated superior root and shoot elongation compared to the control group, whereas electron-dominant plasma (P2) and deionized PAW (PAW1) showed limited growth enhancement.

Table 3: Root and shoot lengths (days 9 and 14), fresh and dry weight, and seedling vigor index of barley seeds under different plasma treatments (mean ± SEM)

| Treatment | Root Length (Day 9, mm) | Shoot Length (Day 9, mm) | Root Length (Day 14, mm) | Shoot Length (Day 14, mm) | Fresh Weight (mg/seedling) | Dry Weight (mg/seedling) | Vigor Index |
|---|---|---|---|---|---|---|---|
| **P1** | 33.3 ± 1.7 | 42. 7 ± 1.2 | 44.0 ± 1.5 | 95.0 ± 2.9 | 29.03 ± 0.17 | 9.07 ± 0.09 | 7082.0 |
| **P2** | 25.0 ± 1.7 | 30.3 ± 2.9 | 27.3 ± 2.3 | 88.7 ± 5.8 | 21.70 ± 0.12 | 7.20 ± 0.06 | 3335.6 |
| **PAW1** | 18.7 ± 0.7 | 29.0 ± 2.1 | 33.0 ± 4.2 | 83.0 ± 1.5 | 23.57 ± 0.13 | 7.53 ± 0.03 | 3824.7 |
| **PAW2** | 34.0 ± 4.0 | 45.0 ± 2.9 | 45.0 ± 3.8 | 86.7 ± 4.3 | 27.07 ± 0.09 | 8.13 ± 0.03 | 5796.9 |
| **Control** | 28.3 ± 3.3 | 31.0 ± 4.0 | 38.3 ± 1.7 | 83.3 ± 6.0 | 22.93 ± 0.09 | 7.47 ± 0.03 | 4295.8 |

On day 9, P1 significantly increased shoot length to 42.7 ± 1.2 mm and root length to 33.3 ± 1.7 mm, followed by PAW2 with 45.0 ± 2.9 mm shoot length and 34.0 ± 4.0 mm root length ($P < 0.01$, ANOVA with LSD test) (Figure 7). These treatments likely enhanced early vigor by etching seed coats and producing RNS, particularly NO, which upregulates nitrate reductase genes and boosts gibberellin production, as reported in [36]. This mechanism promotes metabolic activation and radicle emergence, supporting cell growth and division during seedling establishment [28, 31, 37]. In contrast, P2 and PAW1 showed limited growth enhancement, with P2 reducing root length to 25.0 ± 1.7 mm, indicating oxidative stress from electron-dominant plasma [22].

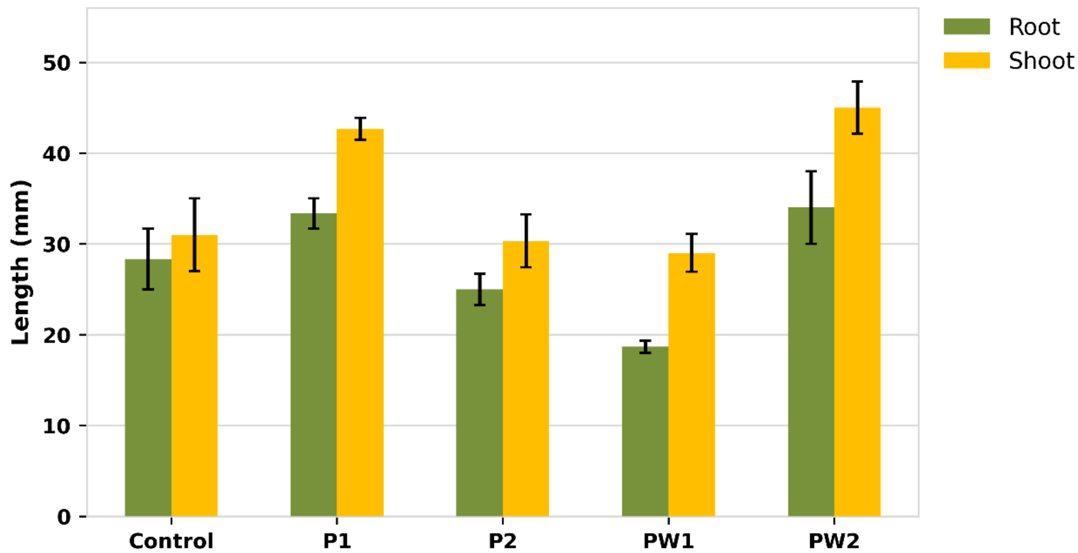


Figure 7: Root and shoot lengths (mm) of barley seedlings on day 9 under different plasma treatments ($P < 0.01$). Error bars represent the standard error of the mean (SEM).

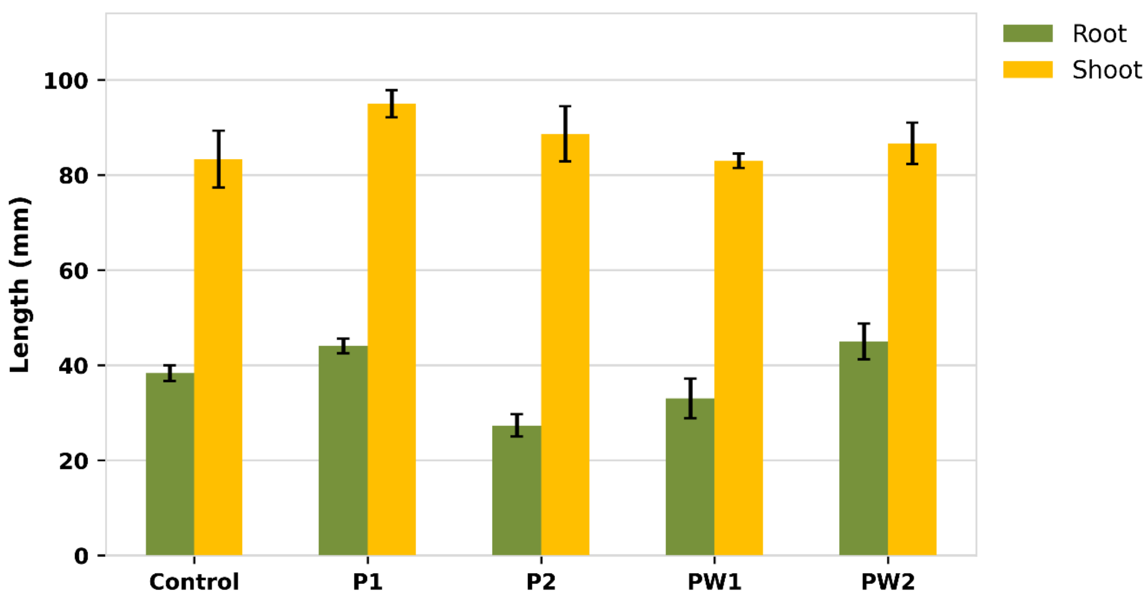


Figure 8: Root and shoot lengths (mm) of barley seedlings on day 14 under different plasma treatments. Error bars represent the standard error of the mean (SEM).

On day 14, both P1 and PAW2 continued to promote root elongation, with measured lengths of 44.0 ± 1.5 mm and 45.0 ± 3.8 mm, respectively (Figure 8). However, shoot lengths showed no significant differences among treatments ($P > 0.05$). Although PAW2 has a positive impact on shoot development and biomass accumulation, its relatively high electrical conductivity (1281 μS/cm) and total dissolved solids (620 mg/L) might cause osmotic stress, potentially limiting further root growth [24]. Therefore, it is essential to optimize the physicochemical characteristics of PAW to mitigate harmful effects on plants and ensure effective results across plant species and environmental conditions [38].

Table 3 also reveals that P1 seedlings achieved the highest biomass, with a fresh weight of 29.03 ± 0.17 mg/seedling (+26.6%) and a dry weight of 9.07 ± 0.09 mg/seedling (+21.4%) compared to the control (22.93 ± 0.09 mg and 7.47 ± 0.03 mg/seedling). PAW2 also increased biomass (fresh: 27.07 ± 0.09 mg/seedling, +18.0%; dry: 8.13 ± 0.03 mg/seedling, +8.8%). In contrast, P2 produced the lowest biomass (fresh: 21.70 ± 0.12 mg/seedling, -5.3%; dry: 7.20 ± 0.06 mg/seedling, -3.6%), reflecting its low vigor index (3335.6). PAW1 yielded intermediate values (fresh: 23.57 ± 0.13 mg/seedling, +2.8%; dry: 7.53 ± 0.03 mg/seedling, +0.8%). These results are likely associated with P1 and PAW2 enhancing water uptake, nutrient mobilization, and metabolic activation through seed coat modifications and RNS generation [4, 13, 23].

### 3.3. Seedling Vigor Index

The seedling vigor index represents an integrated indicator of seed performance by combining germination efficiency with early seedling growth parameters (root and shoot length). In the present study, the vigor index was calculated using measurements obtained on day 9, providing a consistent metric for evaluating early seedling establishment under different plasma treatments. As shown in Table 3, the P1 treatment produced the highest vigor index (7082.0), followed by PAW2 (5796.9), indicating a marked enhancement in seedling performance relative to the control (4295.8). The observed improvements in germination and seedling development may be associated with plasma-induced physiological responses, including potential modulation of enzymatic activity and gene expression pathways; however, these mechanisms were not directly investigated in the present study. Such responses are frequently linked in the literature to RNS generation and plasma-induced surface modifications that enhance water uptake and metabolic activation. In contrast, P2 and PAW1 treatments yielded comparatively lower vigor indices (3335.6 and 3824.7, respectively), suggesting reduced biological effectiveness under these conditions.

The superior vigor index observed for P1 is consistent with previous reports [13, 24, 36, 39] describing plasma-mediated stimulation of physiological processes related to seed dormancy release, membrane permeability, and early growth regulation. Similarly, the enhanced vigor under PAW2 treatment indicates that PAW can promote seedling development when its physicochemical characteristics, particularly pH, EC, and reactive species content, are within biologically favorable ranges. Conversely, the moderate response observed for PAW1, despite its lower pH, highlights the complex nature of PAW efficacy, where water composition, reactive species stability, and potential osmotic effects may collectively influence the final biological outcome.

### 3.4. Statistical analysis

The statistical analysis revealed distinct effects of two nitrogen-based cold plasma treatments, direct glow discharge and indirect PAW, on germination and early growth performance in barley. Significant differences were observed among treatments for germination rate ($P < 0.0001$), root length on day 9 ($P = 0.0099$), shoot length on day 9 ($P = 0.0047$), and root length on day 14 ($P = 0.0073$). However, shoot length on day 14 did not show significant variation between the groups ($P = 0.366$). These results imply that although plasma exerts a positive effect on shoot growth, its influence may diminish over time or be modulated by environmental and physiological factors. Pairwise comparison further indicated that P1 consistently demonstrated better performance across most traits, including seedling vigor, emphasizing its strong biological effect. Overall, these findings confirm that cold plasma, particularly when applied directly through a nitrogen-based glow discharge, can elicit significant physiological reactions in seeds.

### 3.5. Optical Emission Spectroscopy Results

The optical emission spectroscopy of the glow discharge nitrogen plasma and its active species have been illustrated in Figure 9. As seen, the horizontal axis represents the wavelength in the range of 300 to 900 nm, and the vertical axis represents the normalized intensity. The dominant peaks observed in the near ultraviolet and short visible region, especially in the range of 330 to 430 nm, are due to the presence of excited neutral nitrogen species $N_2$ and nitrogen ion $N_2^+$

[40,41]. Radiation in this spectral region, together with active species produced in the plasma, including reactive nitrogen species, can modify the surface chemistry of the seed coat, increase surface energy, increase wettability, and create active functional groups. These surface changes facilitate water uptake, increase seed coat permeability, and accelerate the initiation of metabolic processes associated with germination [42]. The presence of background noise and the relatively large peak width are due to the limited accuracy of the spectrometer and the actual measurement conditions and indicate that the spectrum was recorded under the operating conditions of the device.

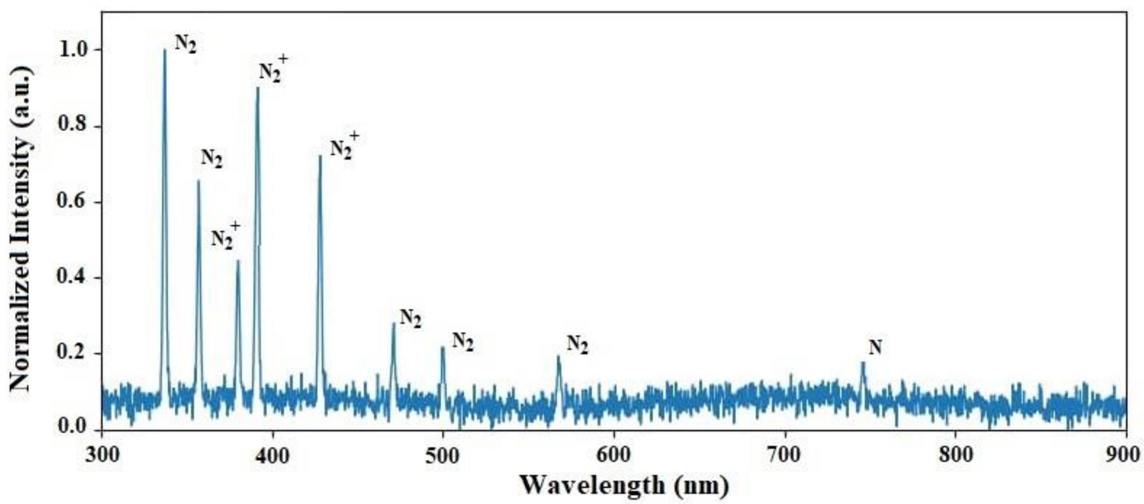


Figure 9: The optical emission spectroscopy of glow discharge nitrogen plasma.

### 3.6. Contact Angle Measurements and Surface Wettability

Water contact angle (WCA) measurements quantitatively demonstrate the progressive enhancement of barley seed surface wettability following ion-dominated nitrogen plasma exposure (Figure 10). Untreated seeds exhibited apparent static WCAs of 85-95° (mean ≈90°), indicative of a moderately hydrophobic native seed coat. This behavior is characteristic of cereal seeds and is commonly associated with the presence of waxes, cutin, and lipid-rich surface layers that limit water penetration and delay imbibition [43]. Ion-dominated plasma treatment induced a clear time-dependent reduction in WCA: ≈65-70° after 1 min (reduction of ~25°), 45-55° after 3 min, and 15-25° after 5 min (overall reduction of 60-75% relative to control), signifying a transition to highly hydrophilic behavior. Comparable monotonic shifts from >80-90° to <30-50° are consistently reported for nitrogen plasma-treated barley, wheat, and other cereals, depending on plasma regime and duration [14,44-48].

This hydrophilization is primarily attributed to energetic ion bombardment, which induces bond scission in organic surface layers, creates reactive sites, and facilitates incorporation of polar functional groups (hydroxyl, carbonyl, amine) through interactions with excited nitrogen species and residual oxygen [49]. Concurrent micro-roughening increases effective surface area, amplifying wettability via combined chemical and topographic effects [45]. Although XPS or FTIR analyses were not conducted, the observed trends align with prior studies linking oxygen enrichment and lipid oxidation to enhanced hydrophilicity in glow-discharge plasma-treated seeds. Functionally, the pronounced hydrophilization after 3-5 min treatments facilitates rapid water imbibition, reduces seed coat resistance to moisture uptake, and promotes earlier metabolic activation, providing a direct mechanistic link to the superior germination rate (93.3%), seedling vigor index (7082.0), and early growth observed under ion-dominated conditions [34,43] (Sections 3.1-3.3). These findings underscore controlled surface wettability modification as a key mediator of nitrogen cold plasma effects on barley seed performance [14, 34].

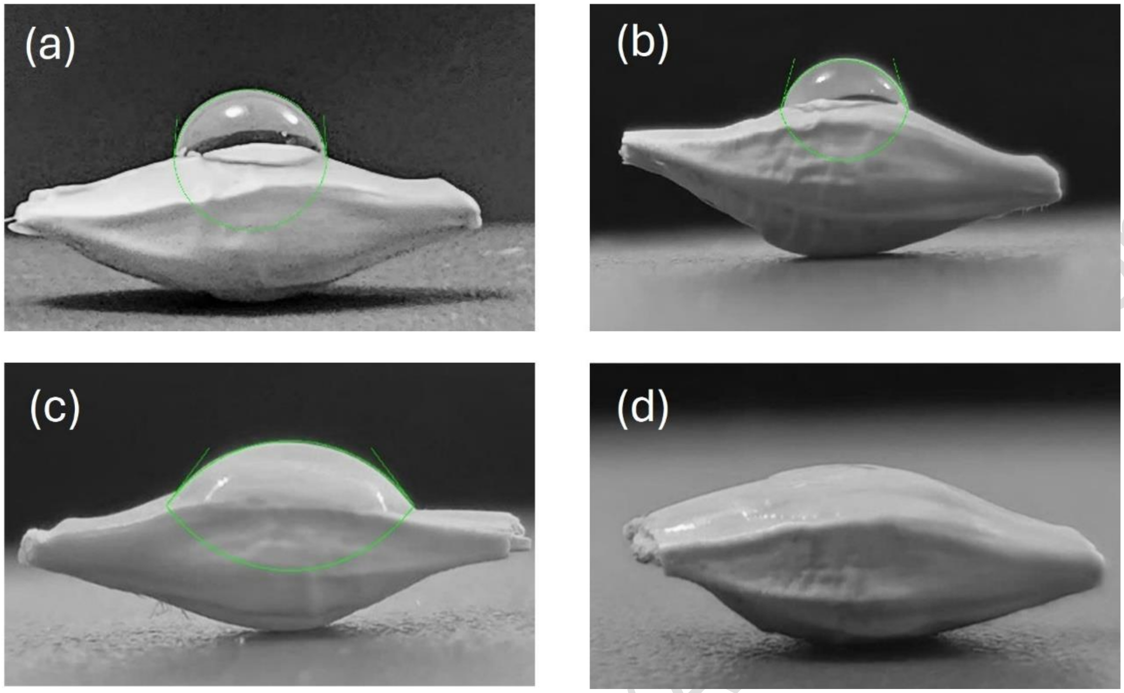


Figure 10: Representative water contact angle images of barley seeds before and after ion-dominated nitrogen cold plasma treatment: (a) untreated control (~85-95°), (b) 1 min treatment (~65-70°), (c) 3 min treatment (~45-55°), and (d) 5 min treatment (~15-25°)

**4. Conclusions**

This research presents a comparative investigation on the germination and seedling growth of barley seeds by two treatment approaches: direct low-pressure nitrogen plasma (glow discharge) and indirect plasma-activated water (atmospheric-pressure DBD). The ion-dominant direct plasma treatment (P1) yielded the most pronounced biological response, with a germination rate of 93.3%, enhanced root and shoot growth, and a seedling vigor index of 7082.0, substantially outperforming both the untreated control (72.0%) and the electron-dominant regime (P2, 60.3%). These superior biological outcomes are mechanistically linked to plasma-induced physicochemical modifications of the seed coat. Optical emission spectroscopy confirmed the presence of excited $\mathrm{N_2}$ and $\mathrm{N_2^+}$ species, with dominant emissions in the 330-430 nm range, while water contact angle measurements demonstrated a clear time-dependent hydrophilization, transitioning from moderately hydrophobic (85-95°) in untreated seeds to highly hydrophilic surfaces (15-25° after 5 min of ion-dominant exposure). Such surface activation promotes rapid water imbibition, reduces resistance to moisture penetration, and facilitates early metabolic initiation, including enhanced enzymatic activity and nitrogen-related signaling pathways.

Indirect treatment via PAW also improved germination and growth, particularly with deionized water (PAW1, 79.7% germination). The low pH of approximately 3.42 was beneficial in stabilizing the reactive nitrogen species. Remarkably, PAW prepared from tap water (PAW2) showed a vigorous early shoot/root elongation and biomass accumulation despite its relatively high electrical conductivity of 1281 µS/cm. This highlights the complex interplay between the chemistry of water and seed physiology.

Despite these promising results, a key limitation remains the qualitative nature of the OES analysis (restricted to a single condition due to instrumental constraints) and the lack of detailed surface chemical characterization (e.g., XPS or FTIR). Future studies should incorporate quantitative plasma diagnostics, multi-parameter optimization across cultivars and environmental stresses, and field-scale validation to enable standardized, scalable treatment protocols. Overall, these findings establish low-pressure ion-dominant nitrogen glow discharge as an effective, chemical-free seed priming strategy that improves surface wettability, alleviates dormancy constraints, and enhances seedling vigor in barley. By highlighting the critical roles of plasma regime (ion- versus electron-dominant) and application mode (direct versus indirect), this work reinforces the potential of cold plasma technology as a sustainable, eco-friendly tool for cereal seed enhancement and resilient agricultural systems.

**Acknowledgement**

The authors would like to thank Mr. M. Bakhshzad Mahmoudi, Head of Plasma Processing Laboratory at the Plasma Physics and Fusion Research School, for his help in performing the experiments.

**Declarations**

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Conflicts of Interest**
The authors declare that there is no conflict of interest regarding the publication of this paper.

**Ethical Approval**
Not applicable.

**Informed Consent**
Not applicable.

**Data Availability**
The data supporting the findings of this study are available from the corresponding author upon reasonable request.

**Authors' Contributions**
S.T. and A.K. conceived and designed the study and performed the experimental work. S.T. analyzed the data and drafted the manuscript with support from A.K. All authors discussed the results, contributed to the review, and approved the final version of the manuscript.

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