



Growth Enhancement of Radish Seed Induced by Low-Temperature Argon Plasma

Rajesh Prakash Guragain¹ · Hom Bahadur Baniya² · Suman Prakash Pradhan³ · Bishnu Prasad Pandey⁴ · Bikash Shrestha¹ · Maciej Fronczak⁵ · Hanna Kierzkowska-Pawlak⁵ · Deepak Prasad Subedi¹

Received: 19 August 2022 / Accepted: 23 September 2022 / Published online: 27 October 2022
© The Author(s) 2022

Abstract

The process of seed germination is described as the culmination of a series of events that start with wetting and end with the emergence of the embryo (typically the radicle) from the seed coat. This method has an impact on crop output and quality. The characteristics of the seed surface and the surroundings have an impact on the kinetics and volume of water absorption by seeds. As a result, modifying the characteristics of a seed's surface is a useful tactic for influencing seed germination. Low-temperature plasma (LTP) treatment of the seed for surface activation is now being researched as an effective pre-sowing technique. This study looked at the effects of LTP on radish (*Raphanus sativus*) seeds at room temperature for various times. The seed's germination properties, growth parameters, water contact angle (WCA), Scanning Electron Micrograph (SEM), and FTIR analysis were examined. WCA and SEM examination revealed a considerable alteration in the seed coat following LTP treatment, which is directly connected to water permeability into the seeds. Similarly, FTIR analysis confirmed that the enhancement of the hydrophilic properties of the seeds following plasma treatment is likely due to the damage of the hydrophobic moieties on the seeds' surface. The LTP treatment improved each germination-related parameter, including the vigor index, chlorophyll content, in-vitro radical scavenging activities, total flavonoid, and phenol in the seedlings. Our findings indicate that LTP treatment has a beneficial impact on early seed sprouting and the development of radish.

Keywords Low-temperature plasma (LTP) · Germination · Seedling growth · Electron density · Excitation temperature · Reactive oxygen–nitrogen species (RONS)

✉ Rajesh Prakash Guragain
rayessprakash@gmail.com

✉ Hom Bahadur Baniya
hombaniya@gmail.com

Introduction

White Radish is the popular name for *Raphanus sativus* around the world, which belongs to the Brassicaceae family and is used in culinary as a dietary supplement. People around the world consume *R. sativus* mainly in the form of salads, vegetables, pickles, and juice [1]. The evidence of traditional medicine practice shows that the various parts of *R. sativus* are used in treating jaundice, gastrointestinal disorders, and liver diseases [2, 3]. Moreover, it contains various vitamins, carbohydrates, sugars, fibers, minerals, and secondary metabolites which have various promising biochemical activities in augmenting good health in human beings [1, 2]. So, *R. sativus* is now of attraction to scientific communities as an ingredient for producing healthy, functional food.

The worldwide population growth is triggering the highest ever-demand food supply and causing environmental exploitation. Ending world hunger is one of the Sustainable Development Objectives, and feeding a growing world population is a major socioeconomic challenge on a global scale. An upsurge in global industrialization, infrastructure development, and urbanization is causing land degradation, loss of productivity, and ultimately affecting food security. To overcome the problems of food security, it is of utmost importance to maintain and increase crop and food production through sustainable, economically viable, and socially feasible modern agricultural processes [4]. Along with land degradation, the loss of genetic diversity, seed damage, excessive use of pesticides, nutrient loss, and slapdash irrigation practice in contemporary agriculture is accelerating the loss of early seed sprouting and development [5]. To overcome the problem of the loss of germination capacity of seeds and the exhausted growth of seedlings, various physical, chemical, and biological methods are in use. Although those methods can accelerate seed germination and improve germination rates, they are labor-intensive, time-consuming, and produce residues that might directly affect the environment [6].

Seed germination is critical and one of the most sensitive processes in the physiological development of plants [7]. The rigorous research in plasma science and its application over the last few decades has provided various evidence that LTP seed exposure can significantly improve early seed sprouting and development [8]. Low-temperature plasma (LTP) is one of the proven methods of enhancing plant cultivation's potential. In addition to accelerating seed germination and improving germination rate, pre-sowing LTP treatment can increase germination, growth, enzymatic activity, and plant yield [9–12]. The pace at which seeds germinate is a measure of how well a crop may be produced overall, and it is affected by water absorption, which causes the embryo's dormant stage to be broken down. The main principle behind these positive impacts of plasma treatment in the seed is basically due to the ability of plasma to produce a chemically rich healthy environment and its ability to enable the delivery of energetic reactive species in a very beneficial way on the surface of biological tissue [13]. Furthermore, the LTP treatment in seeds minimizes surface contamination and stops the spread of undesirable pathogenic microbes [14]. So, the application of plasma is one of the best ways to enhance seed germination and seedling growth of commonly used food crops with no negative environmental impacts [15–17].

Radishes are farmed and consumed worldwide, so a number of studies have been done on their seeds. Following the promotion of crop growth, biomass yield, branching, seed ripening, and better disease resistance, the use of cold plasma has beneficial effects on seed disinfection and sprouting [18]. Tonmitr et al. exposed seedlings to admixture discharge gas of Ar-O₂ and showed that the LF microwave hybrid plasma produced more reactive oxygen species than a traditional LF plasma jet [19]. According

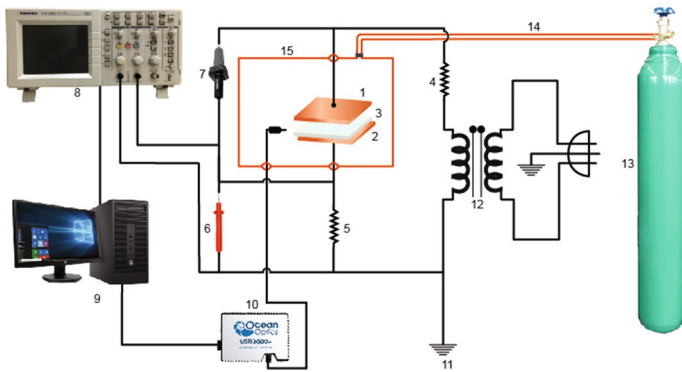
to analysis using SEM, the wettability of the radish seed was modified by etching effects on the seed coat that followed the argon plasma treatments [20]. The kinetics of seed germination was affected by plasma treatment, and the maximum germination rate was influenced by seed color and the length of time the seeds were stored after harvest. The sprouts from grey radish seeds responded more favorably to plasma treatment than those from brown radish seeds [21]. On seedlings that had been infected, plasma reduced *Escherichia coli* and *Bacillus pumilis* by 90% and *Fusarium oxysporum* by 99%. Overall, plasma systems have great potential, but further research is needed [22]. The majority of findings on the effects of seed treatment with NTP come from laboratory or small-scale field research. The effects of NTP on plants should be confirmed in larger-scale agricultural research, and NTP treatment systems for huge amounts of seeds should be developed [23].

For the past few years, Nepal has been facing various challenges in the field of agriculture. The rapid pace of unplanned urbanization and developmental activities, along with an increase in population, triggers and reduces the land availability to conduct agricultural activities [24]. Moreover, the drastic change in the weather pattern, reduced water and fertilizer availability, and declining manpower numbers make it difficult to carry out regular and conventional agricultural activities. However, food demand is increasing rapidly, while agricultural production is decreasing in Nepal, on the other hand [25]. The introduction of contemporary "plasma agriculture" along with intensified farming practice will be one of the greatest alternatives to overcome the problem of low agricultural productivity. Plasma agriculture potentially also has a crucial role in the inactivation of microorganisms while having minimal adverse environmental effects, in addition to accelerating seed germination and improving germination rate. *R. sativus* is a prominent food crop in Nepal. Therefore, in this work, we looked at how LTP can improve early seed sprouting and development of *R. sativus*, as well as how post-treatment affects the physicochemical and biological parameters of seedlings.

Materials and Methods

Details of Experimental Setup

The typical experimental setup employed in the current work is shown in Fig. 1. A translucent polycarbonate cylinder measuring $357 \times 200 \times 150$ mm makes up the reactor chamber. A rectangular copper strip measuring $75.4 \times 49.8 \times 10.0$ mm was used as an electrode. A 2.0 mm thick polycarbonate plate was used as a dielectric barrier between them. During this whole work, a discharge at line frequency was created in an argon environment (4 L/min) using a 12.50 kV high voltage ac power source. A high voltage probe (HVP—28 HF) fabricated by PINTEK with an attenuation ratio of 1000:1 was used to measure the inter-electrode voltage. An oscilloscope probe was used to measure the discharge current across a 10 k Ω shunt resistance. The voltage and current waveforms were recorded by an oscilloscope. USB 2000+ spectrometer was used for the identification of excited species in the discharge. A flow meter was used to regulate the Ar gas flow rate to guarantee the stability of the discharge. At least 250 radish seeds were mechanically positioned into the generated LTP discharge to maintain uniform treatment. Seeds were exposed to LTP for periods varying from 1 to 3 min during the trials.



Specification: “(1,2) Electrodes, (3) Dielectric Sheet (PC), (4) Ballast Resistor, (5) Shunt Resistor, (6) Current Probe, (7) High Voltage Probe, (8) Oscilloscope, (9) Computer Interface, (10) OES, (11) Ground, (12) High Voltage Transformer, (13) Gas Cylinder, (14) Pipe, (15) Reaction Chamber”

Fig. 1 Experimental arrangement

Seed Collection and Growing Condition

The “Nepal Agricultural Research Council (NARC), Lalitpur, Nepal” provided the *R. sativus*. for the investigation. Instead of hybrid seeds, only locally produced seeds were utilized. Radish seeds that were in good condition and had no noticeable flaws were chosen for germination. On the bottom electrode in the discharge zone that was covered by the dielectric, 250 radish seeds were mechanically positioned into the generated LTP discharge to maintain uniform treatment. The seeds were then treated for 1–3 min using the created discharge. The seeds were immediately kept in sterile jars upon treatment and stored there until they were not sown. The cocopeat was cleaned with distilled water and then dried at room temperature. Cocopeat was then added to a germination tray. To ensure the accuracy of the results, each tray had three replicates of each control and treated 250 radish seeds, and the germination parameters were studied on various germination days. An equal amount of distilled water was used every two days on the tray to maintain the cocopeat’s moisture level to avoid evaporation-related alterations.

Study of Growth Characteristics

Estimation of Germination Percentage (G %)

Germination percentage is a measure of the survival of a collection of seeds [26].

$$\text{Germination percentage} = \frac{\sum_{i=1}^k n_i}{N} \times 100 \quad (1)$$

where N is the total number of seeds utilized, and n_i is the number of seeds that germinated on the i th day.

Relativized Percentage of Germination (R %)

As indicated in Eq. 2, “relativize germination percentage enables comparisons between treatments that are equivalent when the quantity of dormancy disrupts varies” [27].

$$R\% = \frac{\text{Actual percentage}}{\text{The highest percentage among the group of datas}} \times 100 \tag{2}$$

The Mean Germination Time (MGT)

MGT as expressed in Eq. 3 is defined as “the average time it takes for a seed to germinate or emerge” [27, 28].

$$\bar{t} = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i} \tag{3}$$

where $n_i t_i$ is the number of seeds germinated on the i th interval, and n_i is the number of seeds germinated on the i th time.

The Mean Germination Rate (MGR)

“MGR was calculated as the reciprocal of the MGT” [28].

$$\bar{v} = \frac{1}{\bar{t}} \tag{4}$$

where \bar{t} is the mean germination time.

Uncertainty of the Germination Process (U)

“The germination process uncertainty, which is determined by Eq. 5, indicates the degree of uncertainty associated with the relative frequency of germination dispersion” [27].

$$U = \sum_{i=1}^k f_i \log_2 f_i \tag{5}$$

$$f_i = \frac{n_i}{\sum_{i=1}^k n_i}$$

where f_i represents the relative frequency of germination.

Synchrony of Germination Process (Z)

“It specifies the amount to which members in a certain demographic overlap. The synchronization index produces a value if and only if two seeds finish the sprouting process in the same moment” [29]. It can be estimated using Eq. 6.

$$Z = \frac{\sum_{i=1}^k C_{n_{i,2}} t_i}{C_{\sum n_{i,2}}} \quad (6)$$

$$C_{n_{i,2}} = \frac{n_i(n_i - 1)}{2}$$

where $C_{n_{i,2}}$ = combination of seeds germinated in the i th time, two by two, and n_i is the number of seeds germinated on i th time. The number Z equals one when all seeds sprout at the same time. And when Z is zero, at least two seeds may sprout simultaneously.

Coefficient of Variation of Germination Time (CV_t)

Equation 7 was employed to estimate the (CV_t) [29].

$$CV_t = \frac{S_t}{\bar{t}} \times 100 \quad (7)$$

$$S_t = \sqrt{\frac{\sum_{i=1}^k n_i(t_i - \bar{t})^2}{\sum_{i=1}^k (n_i - 1)}}$$

here S_t represents the standard deviation of germination time and \bar{t} represents MGT.

Germination Index (GI)

“The germination index is a measurement of how long it takes for a specific percentage of seeds to germinate (in days)” [30].

$$GI = \frac{\sum_{i=1}^k n_i}{t_i} \quad (8)$$

where the symbols have their usual meanings.

Coefficient of Velocity of Germination (CVG)

CVG was measured by using Eq. 9 [31].

$$CVG = \frac{\sum_{i=1}^k n_i}{\sum_{i=1}^k n_i t_i} \times 100 \tag{9}$$

Time to 50% Germination (T₅₀)

“T₅₀ is the time required for 50% of the seeds to germinate” [30].

$$T_{50} = \frac{t_i + \left(\frac{\sum_{i=1}^k n_i}{2} - n_i \right)}{n_j - n_i} (t_j - t_i) \tag{10}$$

To obtain the values of n_i and n_j in above equation, we examine the total number of seeds sprouted, which is listed as a criterion below.

$$n_i < \left(\frac{\sum_{i=1}^k n_i}{2} \right) < n_j$$

where n_i = nearest cumulative number of seeds germinated $C_{n_i} < \left(\frac{\sum_{i=1}^k n_i}{2} \right)$.

n_j = nearest cumulative number of seeds germinated $C_{n_j} > \left(\frac{\sum_{i=1}^k n_i}{2} \right)$.

t_i = the time corresponding to n_i .

t_j = the time corresponding to n_j .

Similarly, time-related germination characteristics, like T₁₀, T₂₅, T₇₅, and T₉₀ can be estimated simply by changing $\frac{\sum_{i=1}^k n_i}{2}$ with $\frac{\sum_{i=1}^k n_i}{10}$, $\frac{\sum_{i=1}^k n_i}{4}$, $\frac{3 \sum_{i=1}^k n_i}{4}$ and $\frac{9 \sum_{i=1}^k n_i}{10}$ respectively.

Mean Daily Germination (MGD) Percent

“It is the daily average of how many seeds germinate. The ratio of the number of seeds germinating every day to the total number of seeds germinated is another way to look at it” [32].

$$MDG = \frac{GP}{T_n} \tag{11}$$

here GP represent the final cumulative germination percentage and T_n indicates the total number of intervals required for final germination.

Germination Value

“Germination value is calculated by integrating the speed and completeness of germination into a composite score” [33].

$$G - \text{Value} = \text{MDG} \times \text{PV} \quad (12)$$

here PV = the overall number of germinated seeds at the point on the germination curve when the rate of germination begins to decline.

Statistical Tools

A minimum of three replicates were conducted for each experiment, and the results were calculated as mean \pm standard deviation. Different letters (a–d) among the group denote the significant differences between the mean at $p < 0.05$ using one-way analysis of variance (ANOVA) and Tukey’s multiple comparison test.

Results and Discussion

Electrical Signal Analysis

The typical waveforms of the applied voltages and the associated discharge current in argon are shown in Fig. 2. The total charge transported over a single period is zero, and the curve resembles a parallelogram. It is noticeable that within a single voltage pulse, there are multiple prominent discharge current peaks. An attribute that sets atmospheric pressure DBD apart is the existence of micro-discharge [34].

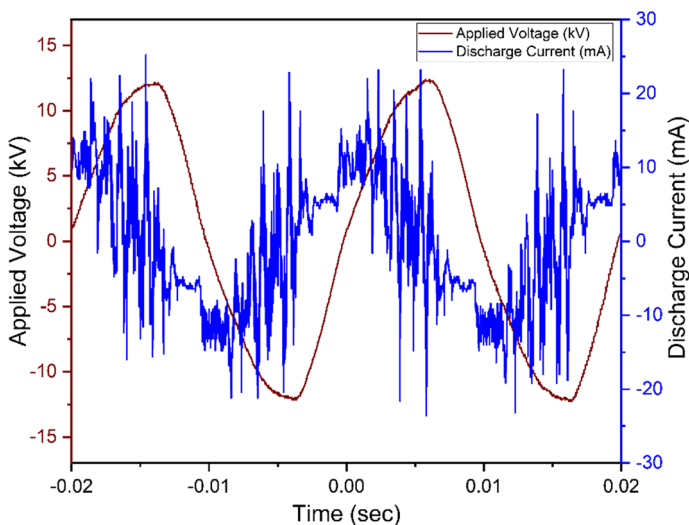


Fig. 2 Waveforms of the discharge currents and applied voltages in argon

When an electric charge (Q) is plotted as a function of the applied voltage (V), Lissajous figure is obtained as indicated in Fig. 3. Similarly, the relation between capacitance of dielectric and air space is given by Eq. 13 [35].

$$\frac{1}{C} = \frac{1}{C_g} + \frac{1}{C_d} \tag{13}$$

where C_d and C_g denotes the capacitance of dielectric and air space respectively.

The expression for the average energy and power consumed [35–37]

$$E = 4C_d \frac{1}{1 + \frac{C_g}{C_d}} V_{\min}(V_{\max} - V_{\min}) \tag{14}$$

$$P = 4fC_d \frac{1}{1 + \frac{C_g}{C_d}} V_{\min}(V_{\max} - V_{\min}) \tag{15}$$

where f is the input signal frequency, V_{\max} and V_{\min} are the maximal and minimal input voltage required to begin the argon discharge respectively.

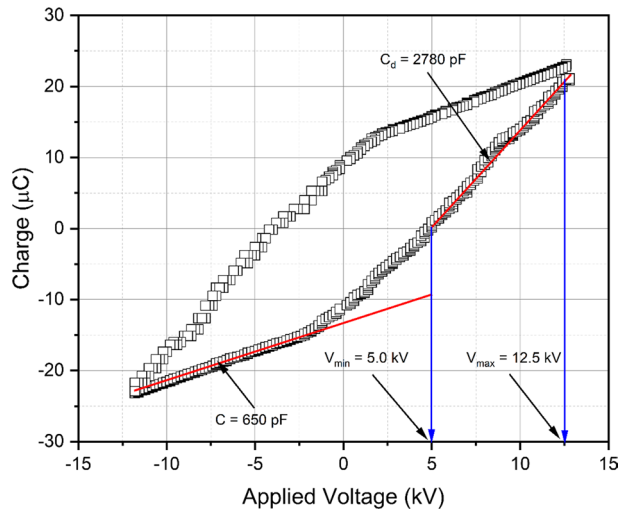
The value obtained from Fig. 3 was kept in Eqs. 13–15, and after calculating, we found that the average energy consumed and the power dissipated were 0.32 J and 15.92 W, respectively. Additionally, we also realized that inserting seeds for treatment in the discharge zone alters the values of these two parameters.

Similarly, discharge voltage (V_D) was obtained from the measured value of V_{\min} , C_g , and C_d [37].

$$V_D = V_{\min} \frac{1}{1 + \frac{C_g}{C_d}} \tag{16}$$

The discharge voltage in our case was determined to be 3.83 kV. The electric field in the discharge zone was found to be 15.3 kV/cm using these values.

Fig. 3 Charge versus voltage diagram of the generated discharge



Similarly, Eq. 17 is used to calculate the electron density (n_e) [38, 39]:

$$n_e = \frac{J}{e\mu_e E} \quad (17)$$

Here J represents the average current density, e the electronic charge, μ_e the electron mobility and, E represents the electric field in the discharge region.

The electron mobility was estimated using Bolsig + software and was found to be 337.56 cm²/Vs. The average electron density was found to be 1.2×10^{11} per cm³ using the obtained values in Eq. 17.

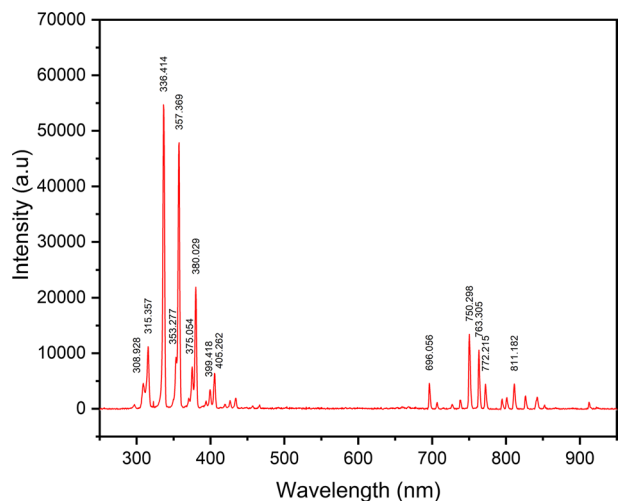
Optical Characterization

For the estimation of excitation temperature, Boltzmann Plot method, was used [40]. Figure 4 depicts the optical emission spectra of an atmospheric pressure LTP argon discharge. The emission spectrum includes Ar lines in the UV and visible-NIR regions as well as OH bands resulting from the dissolution of atmospheric water vapor. The spectrum also includes bands in the UV spectral region of the first negative system of N₂⁺ and the second positive system of N₂ [40, 41]. From the discharge spectrum, eleven suitable lines of Ar I (801.48 , 811.53 , 810.37 , 852.14 , 696.54 , 420.06 , 426.62, 434.51, 706.87, 772.42m, 419.1 nm) were chosen.

$$\text{Ln} \left(\frac{I\lambda_{ji}}{hcA_{ji}g_j} \right) = -\frac{E_j}{KT_{\text{exc}}} + C \quad (18)$$

The wavelength (λ) and intensity (I) of the spectral lines are taken from the observation in Eq. 18. The essential values: g_j (values of statistical weight), A_{ji} (transition probability), and E_j (energy) in Eq. 18 of eleven chosen lines are determined using “The National Institute of Standards and Technology (NIST) Atomic Spectra Database” [42]. Furthermore, K denotes the Boltzmann constant, and C is a constant.

Fig. 4 OES of an atmospheric pressure LTP argon discharge



Finally, plugging energy (E_j) along x-axis and $\text{Ln}\left(\frac{I\lambda_{ji}}{hcA_{ji}g_j}\right)$ along y- axis yields a straight line, and the slope of the straight line can be used to calculate the excitation temperature. As seen in Fig. 5, the excitation temperature is found to be 8526 K. The linear fitting was consistent with an R-square value of almost 0.93, indicating excitation equilibrium of LTP discharge had been satisfactorily verified.

Influence of LTP on Germination parameters

Germination (%) was observed from the 2nd day of seed sowed till the 6th day. Overall, LTP-treated seedlings germinated at a greater rate (%) than untreated seeds. Comparing the 1, 2, and 3- minutes seedlings to control seedlings on the 2nd day, the germination percentage for each group rose by 9.6, 35.6, and 36.8%, respectively. Similarly, the germination percentage of LTP exposed seeds was found to be 15.6, 27.2, and 29.8% higher than the control on the 3rd day after germination. Furthermore, on the 4th day, LTP-exposed seedlings germinated faster than the seed that was not treated. Similarly, on the 5th, 6th, and 7th days after germination, LTP-exposed seeds had a 4% higher germination rate than control seedlings. These findings showed that plasma-exposed seeds germinated more quickly [Fig. 6].

From Table 1, it was clear that there is a significant change in the MGT and MGR before and after LTP treatment. However, no significant differences were observed in 2 and 3 min of treatment time. Our findings are in good agreement with the reported articles by various researchers [28, 43]. Additionally, it has been found that seeds exposed to LTP for 2 and 3 min had a significantly reduced value of the uncertainty process. “Low uncertainty values indicate frequencies with few peaks (i.e., are focused germination over time)”. Its value being close to zero indicates coordinated germination[27, 29]. It was observed that there is no discernible synchronization of germination among untreated and LTP exposed seeds, as shown in Table 1.

The CV_i between control and LTP exposed seeds differs significantly, which can be seen in Table 2. CV_i and GI values was discovered to rise by (10.4, 22.8, and 14.57%) and (25.97, 45.45, and 46.92%) in the case of seeds exposed to LTP for 1, 2, and 3 min, respectively when compared to seeds that were not exposed to LTP, However, 2 and 3-min LTP

Fig. 5 Linear fitting of Boltzmann plot’s data to determine excitation temperature

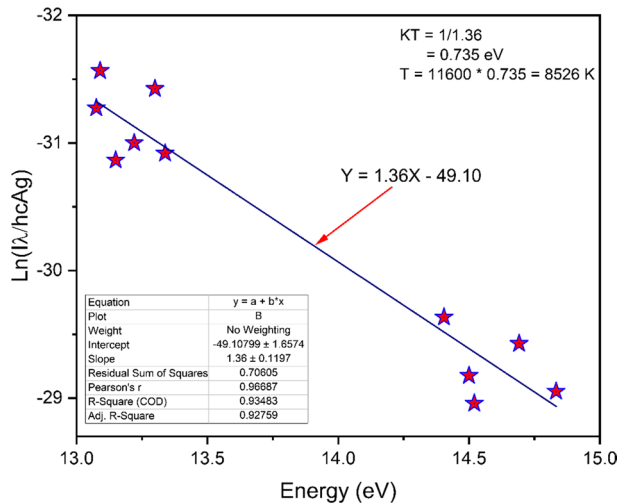


Fig. 6 Estimation of germination percentage after LTP treatment

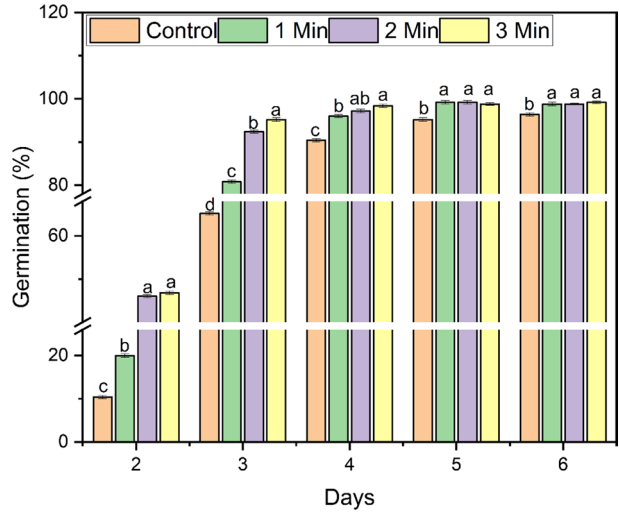


Table 1 Estimation of “Relativized Percentage of Germination (RPG), Mean Germination Time (MGT), Mean Germination Rate (MGR), Uncertainty of Germination Process (*U*), and Synchrony Germination Process (*Z*)” before and after LTP treatment

| Treatment Time | RPG (%) | MGT (day) | MGR (day) ⁻¹ | <i>U</i> (bit) | <i>Z</i> |
|----------------|----------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Control | 97.00 ± 1.00 ^b | 3.71 ± 0.01 ^a | 0.27 ± 0.01 ^c | 1.44 ± 0.01 ^b | 0.49 ± 0.00 ^a |
| 1 min | 100.00 ± 1.00 ^a | 3.02 ± 0.01 ^b | 0.33 ± 0.00 ^b | 1.47 ± 0.01 ^a | 0.44 ± 0.00 ^c |
| 2 min | 100.00 ± 1.00 ^a | 2.63 ± 0.02 ^c | 0.38 ± 0.00 ^a | 1.35 ± 0.01 ^c | 0.43 ± 0.00 ^c |
| 3 min | 100.00 ± 1.00 ^a | 2.58 ± 0.03 ^c | 0.38 ± 0.00 ^a | 1.24 ± 0.01 ^d | 0.46 ± 0.00 ^b |

Different letters (a–d) among the group denote the significant differences between the mean at *p* < 0.05 using one-way analysis of variance (ANOVA) and Tukey’s multiple comparison test

Table 2 Estimation of “Coefficient of Variation of Germination Time (CV_t), Germination Index (GI), Coefficient of Velocity of Germination (CVG), Mean Daily Germination Percent (MDG), and Germination Value (G-value)” before and after LTP treatment

| Treatment Time | CV _t (% seed/day) | GI (day) | CVG (%) | MDG (%) | G-value |
|----------------|------------------------------|---------------------------|---------------------------|---------------------------|----------------------------|
| Control | 20.86 ± 0.07 ^d | 68.88 ± 0.1 ^d | 26.93 ± 0.07 ^d | 16.08 ± 0.01 ^b | 155.02 ± 0.84 ^d |
| 1 min | 23.05 ± 0.07 ^c | 86.77 ± 0.1 ^c | 33.18 ± 0.03 ^c | 16.54 ± 0.01 ^a | 190.23 ± 0.85 ^c |
| 2 min | 25.62 ± 0.07 ^a | 100.17 ± 0.1 ^b | 38.07 ± 0.02 ^b | 16.54 ± 0.01 ^a | 217.4 ± 1.18 ^b |
| 3 min | 23.9 ± 0.07 ^b | 101.2 ± 0.1 ^a | 38.73 ± 0.03 ^a | 16.54 ± 0.01 ^a | 225.15 ± 0.42 ^a |

Different letters (a–d) among the group denote the significant differences between the mean at *p* < 0.05 using one-way analysis of variance (ANOVA) and Tukey’s multiple comparison test

expose seeds showed no significant differences in GI value. “A higher GI score indicates a higher percentage and rate of germination” [44].

The CVG is “a measure of how quickly seeds germinate and its value rises as the number of germinated seeds rises, and the time needed for germination falls” [31]. From Table 2, we

noticed for 1, 2, and 3 min treated seeds, CVG increased by 23.90, 41.36, and 43.70% and G-value increased by 22.71, 40.23, and 45.23% respectively, when compared to control seeds. We noticed that there was no significant change in MDG between the control and LTP- treated seeds while significant differences were observed in G-value between LTP-treated seeds.

The results from Table 3 showed that LTP-treated seed germinates much more quickly than untreated seed. From germination to ripening through maturation, RONS are engaged in several seed processes [45][45]. RONS is used in a variety of signaling processes throughout the plant life cycle, including the emergence of dormancy during seed germination [47]. Exogenous RONS has been shown to facilitate sunflower and maize seed germination by enhancing the production of gibberellic acid (GA) and the degradation of abscisic acid (ABA) [46]. Additional research has revealed that GA stimulates the germination of dormant caryopses via regulating the levels of ABA and the ROS-antioxidant state [48]. Therefore, as reported by numerous researchers, the results of our research also demonstrate that RONS created in the discharge may cause GA to improve seed germination.

Mass Loss

The percentage of mass loss after LTP treatment is calculated using Eq. 19 [6]:

$$\text{Mass Loss (\%)} = \left(\frac{m_i - m_0}{m_i} \right) \times 100 \quad (19)$$

where m_i = mass of the sample before treatment and m_0 = mass of the sample after treatment.

50 seeds were exposed to LTP for different time interval. The LTP-exposed seeds for 1 min resulted in the highest mass loss and there was a significant difference across treatments, as shown in Fig. 7. The LTP may have etched the seed surface and reduced the treated sample's mass [49]. However, as compared to 1- minute treatment, mass loss is found to be fairly minimal as treatment time rises. Reactive species may have been deposited on the seed surface during the time of treatment thereby resulting in lower mass loss as compared to 1- minute treatment [50].

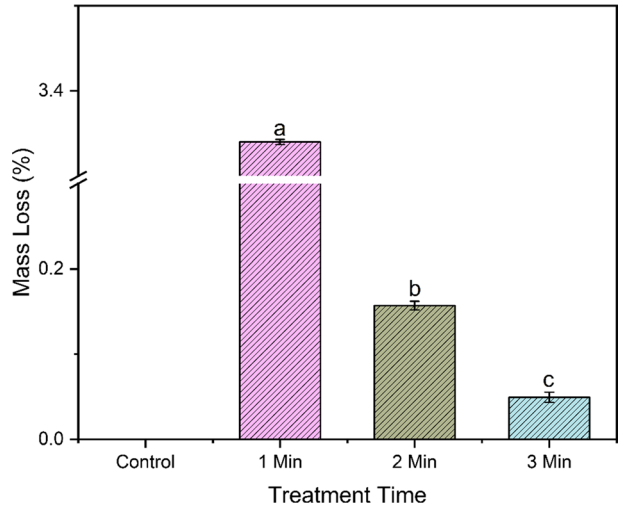
Wettability after LTP Treatment

50 seeds were placed in the LTP discharge area for specified treatment time. The seeds' mass was promptly measured using a weighing machine (MG124Ai) fabricated by Bel instruments. 20 mL of distilled water were kept in a petri dish and then untreated and LTP treated seeds were dipped. Their mass was then measured every 1.5 h for a total of 12 h. Then, the wettability was calculated accordingly using Eq. 20 [51, 52].

Table 3 Estimation of “Time to T_{10} , T_{25} , T_{75} , and T_{90} germination” before and after LTP treatment

| Treatment Time | T_{10} (day) | T_{25} (day) | T_{50} (day) | T_{75} (day) | T_{90} (day) |
|----------------|----------------|----------------|----------------|----------------|----------------|
| Control | 1.93 | 2.93 | 3.35 | 3.72 | 3.94 |
| 1 min | 1.5 | 2.08 | 2.49 | 2.89 | 3.56 |
| 2 min | 1.22 | 1.54 | 2.08 | 2.61 | 2.93 |
| 3 min | 1.21 | 1.53 | 2.06 | 2.57 | 2.88 |

Fig. 7 Estimation of Mass Loss (%) after LTP treatment

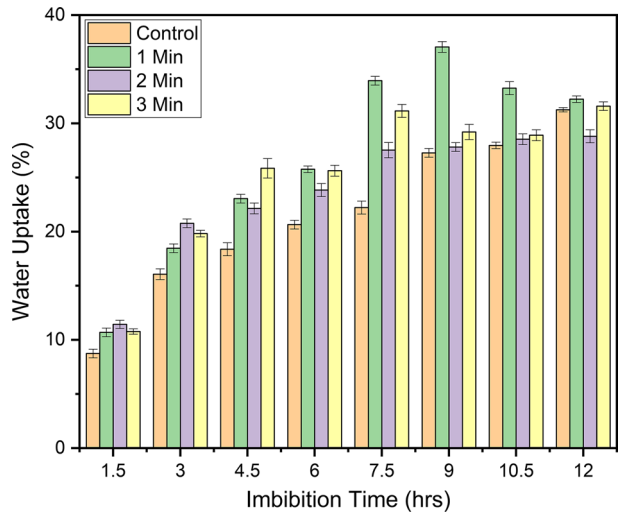


$$\text{Wettability} = \frac{m_i - m_0}{m_0} \times 100\% \tag{20}$$

where m_0 and m_i are the mass before and after they have been dipped in water respectively.

From Fig. 8, we can conclude that after LTP treatment, the water absorption by seeds gets accelerated and is found to be positively correlated with treatment time. The most significant difference at 1 min was seen at 9 h when seeds absorbed 15.28% more water than untreated seed.

Fig. 8 Estimation of water uptake of seeds before and after LTP treatment



Estimation of the Seedling length

From day 7 to day 13, germinated seedlings were carefully removed from the tray and measured with a ruler to measure the total length of roots, shoots and seedling lengths.

Figure 9A illustrates a photograph of the total seedling length taken on the 7th day after seed sowing, while Fig. 9B, C depict the overall seedling length as well as the root and shoot lengths on different days. Compared to control, seedling length increased by 32.78, 39.74, and 64.55% on the 7th adays, whereas it increased by 13.98, 33.64, and 42.57% on the 13th day for 1, 2, and 3-min LTP treated seeds respectively.

Similar results were seen on the 7th day, with shoot length increasing by 61.46, 59.2, and 94.11%, and root length increasing by 6.15, 21.67, and 37.10% for seeds treated for 1, 2, and 3 min, respectively. Similarly, on the 13th day, following 1, 2, and 3 min of plasma treatment, the shoot length increased significantly by 18.9, 50.85, and 57.41%, while the root length increased considerably by 8.54, 14.9, and 26.31% compared to control. Based on our observation, we noticed that the root and shoot lengths as well as the overall total length were found to be considerably larger in the LTP-treated seeds.

Calculation of Vigor Index

The vigor indicates "the totality of the seed's qualities that govern the potential degree of activity and performance during germination and seedling emergence" and is calculated using Eq. 21 [26].

$$\text{Vigor Index I (VI - I)} = \frac{\text{Average seedling length (cm)} \times \text{Germination (\%)}}{100} \quad (21)$$

Plasma treatment significantly increased the vigor index I [Fig. 10]. Compared to the seeds that weren't exposed to LTP, the VI-I of treated seeds improved by 1.3–1.7 times. These may be influenced by a significant rise in seedling total length and fresh weight which is consistent with the findings of several studies [53, 54] for soybean. The variations in germination and early seedling growth noted in our experiment may be explained by the activation of natural signals, hormones, and enzyme activities that result from the direct interaction between LTP and vegetal cells [55].

Estimation of Contact Angle and Surface Free Energy of Seed Surface

The wettability and subsequently the permeability of the seed surface were assessed by measuring water contact. Because a hydrophilic surface absorbs more water to initiate germination than a hydrophobic surface, an increase in hydrophilicity, as shown by a decrease in WCA on the seed surface, is crucial in enhancing seed germination [56, 57].

Figure 11 depicts the change in water contact angle (WCA) and surface free energy (SFE) on untreated and LTP exposed seeds. 2 μL of distilled water was kept to the surface of the seeds for the estimation of WCA. The experiments were repeated three times and then the average value of the WCA and SFE was determined. WCA of the untreated seed was found to be $107.75^\circ \pm 3^\circ$. The contact angle of the seeds reduced to $39.7^\circ \pm 2.1^\circ$, $41.5^\circ \pm 2.8^\circ$, and $44.3^\circ \pm 2.9^\circ$ after 1, 2, and 3 min of LTP treatment.

Fig. 9 A Photograph of seedlings, Estimation of **B** Seedlings length **C** Shoot and root length on different days before and after LTP treatment

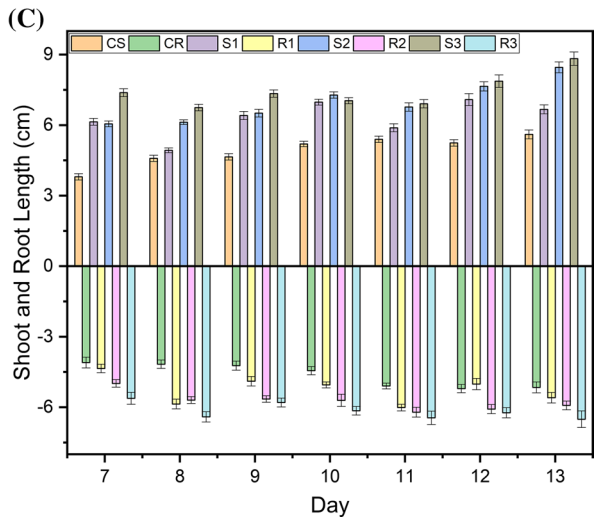
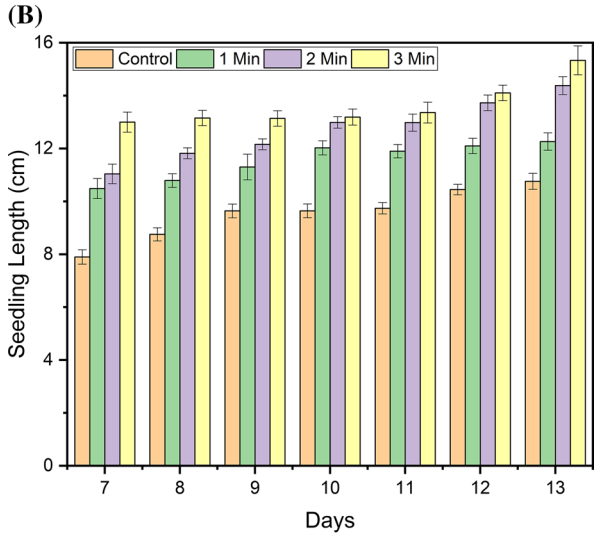
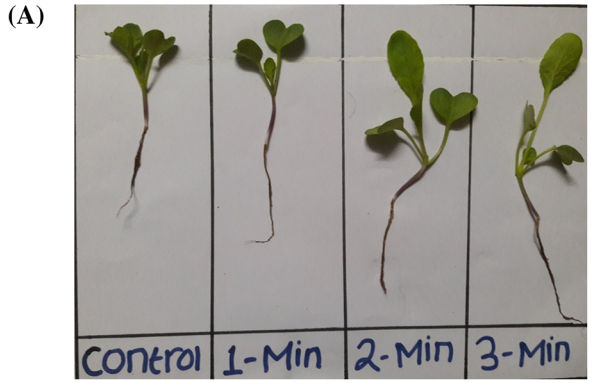


Fig. 10 Estimation of VI-I before and after LTP treatment

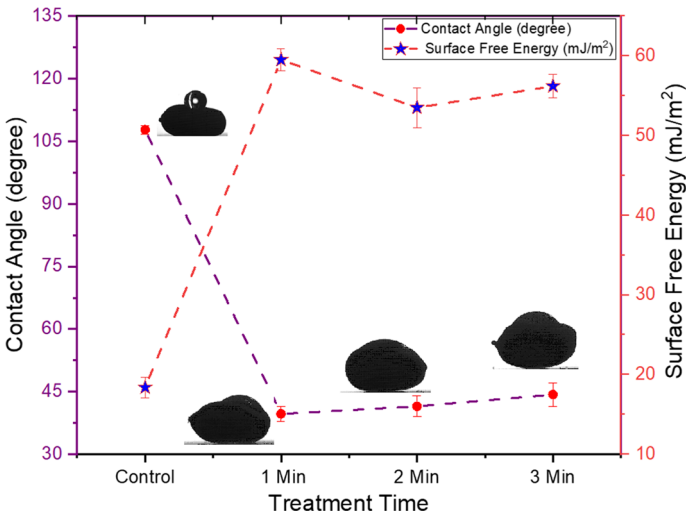
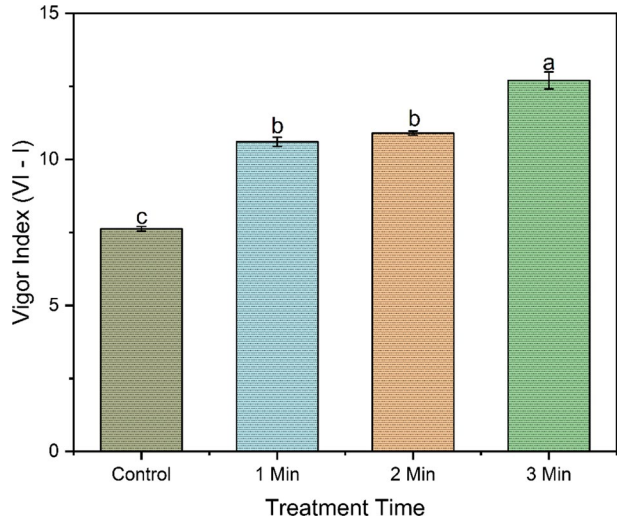


Fig. 11 Estimation of contact angle and surface free energy before and after LTP treatment

Similarly, a considerable variation in surface-free energy was noticed between treated and control seeds.

After LTP exposure, the seed sample had been found to improve water absorption as a result of the decrease of contact angle and established new chemical bonds on the surface, and perhaps functionalize the seed coat. It is hypothesized that interactions between seeds and reactive plasma species of LTP alter not only the characteristics of the seed coat but also the physiology of germination in many plant species [58]. As a result, RONS produced during discharge may be converted to hydrogen peroxide through further interactions with water vapor molecules, which are then ingested and has a favorable impact on metabolic processes. According to our observations,

plasma-treated seeds had significantly improved wettability compared to untreated seeds, which may have accelerated germination.

Scanning Electron Microscope (SEM) Imaginary of Seed's Anatomy

Figure 12 illustrates the SEM images of control and treated seeds exposed to LTP for 1, 2, and 3 min captured using “a scanning electron microscope (JEOL JSM-7001F)”. It is observable that the texture of treated seed is relatively rougher than the control.

Plant seeds are only subjected to extremely mild stress as a result of plasma activation during the LTP treatment, and they remain intact. These characteristics allow the plasma to either enrich the seed's surface with functional groups containing oxygen or produce slight surface etching (via ion bombardment) [59]. These groups can significantly improve the hydrophilicity of the surface, which greatly enhances seed germination and metabolism as well as improves significantly surface permeability for various supplements [43, 53].

FTIR Analysis

The FTIR spectra have been recorded from the one spot on the surface of the seeds before and after the treatment with argon plasma for 5 min. The samples were attached

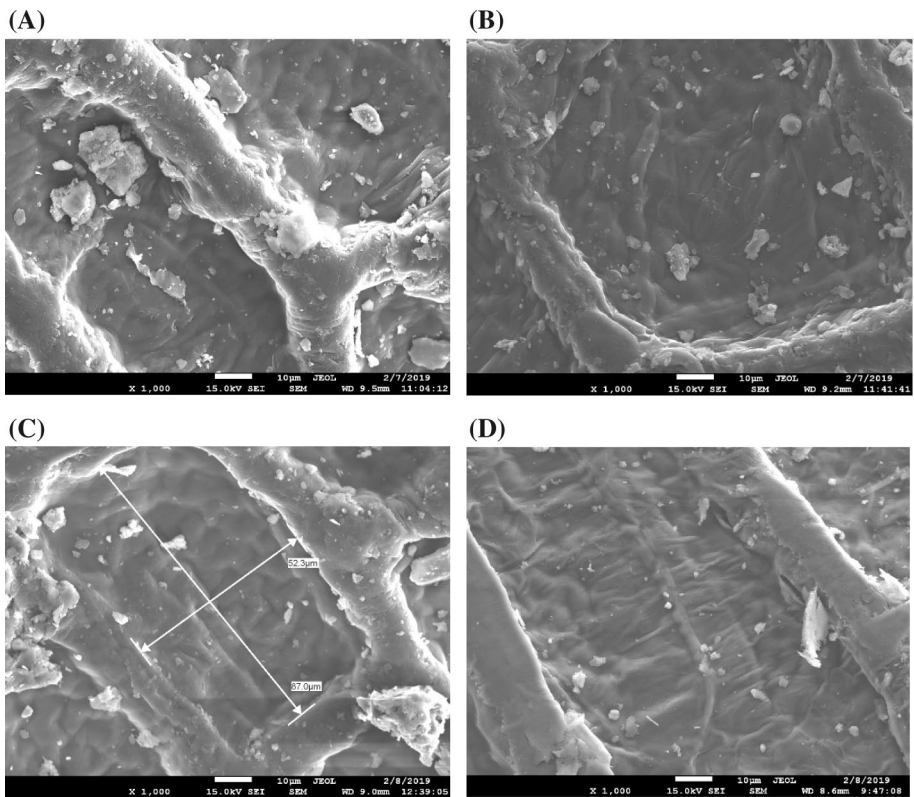


Fig. 12 SEM micrograph of **A** Control, and plasma-treated seed for **B** 1 min, **C** 2 min, and **D** 3 min

with the carbon tape to the silicon wafer. The reflective mode has been applied to the measurement. The Jasco 6400 spectrometer equipped with a microscope and liquid nitrogen—cooled MCT detector has been applied. The magnification of 8 times was applied to focus the IR beam onto the sample. The spectra were recorded in the wavelength range between 4000 and 1000 cm^{-1} with a resolution of 4 cm^{-1} . The spectra were registered in the amount of 1500 and were averaged. It should be noted however that the plasma treatment causes the changes at depths less than 10 nm, whilst the FTIR analysis in reflectance mode is able to analyze the depths between a few hundred nanometers up to few micrometers. Therefore, the FTIR spectra are dominated by the signal collected from the untreated region of the radish seed peel.

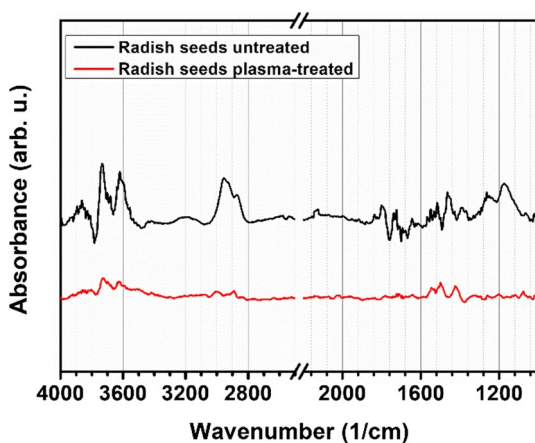
Region between 2500 and 2200 cm^{-1} is not shown, because of the presence of dominating band for CO_2 .

In Fig. 13, we can see that the plasma treatment caused the weakening of the bands characteristic for C-H bonds stretching in the range between 2860 cm^{-1} and 3000 cm^{-1} [60, 61] as well as the band characteristic for C-H scissoring mode at 1470 cm^{-1} [60, 61]. Therefore, the enhancement of the hydrophilic properties of the seeds after the plasma treatment can be tentatively related to the damaging of the hydrophobic moieties on the surface of the seeds.

Plant Collection and Extract Preparation

Leaf samples for biochemical analysis were collected at day 70 of germination. 5 g of the root samples were dried at room temperature and softened with 80% Methanol (50 mL) and 50% Ethanol (50 mL) for 24 h and then Whatman filter paper was used to filter the extract to assess the change in flavonoids, total phenolics, and radical scavenging activities. Using a “vacuum evaporator (Hanil/Modul 4080C)”, the sample was dried. Prior to further examination, the crude samples obtained were kept at 4 °C.

Fig. 13 FTIR spectra of radish seeds: native (black) and plasma-treated (red) (Color figure online)



Estimation of Chlorophyll Content and Total Carotenoids Before and After LTP Treatment

Plant pigmentation is the basis for the photosynthetic transformation of solar energy to chemical energy in plants. Among the various pigments, chlorophyll a, chlorophyll b, and carotenoids plays important role in the physiological processes of plants [62]. In addition, chlorophyll pigments in plant are also considered as an indicator of response to stress and habitat. In general, chlorophyll provides green pigmentation and carotenoids are responsible for red, yellow, and orange pigmentation which are essential for the health of plants [63]. The examination of plant pigments is a good source for the study of plant physiological condition and their health.

As indicated in Table 4, standard protocols were used to measure chlorophyll and carotenoid concentrations in acetone solvent [64].

So, in this study, we evaluated and compared the chlorophyll and carotenoid content of seedling's leaf extracts which were grown by treating seeds with plasma. Our results revealed the remarkable change in chlorophyll-a & b content and total carotenoid content in leaf extracts of untreated and plasma-treated seeds. The highest content of chlorophyll-a was found in 2 min treated seedling leaf extract. The changes in the content of chlorophyll-a in the extract of untreated and seedlings that were treated provide us evidence that the plasma treatment helps in augmenting the total biochemical and physiological activities of seedlings [26, 65]. However, there were no such remarkable changes in chlorophyll-b and carotenoid content due to the LTP treatment [Fig. 14].

Estimation of Total Flavonoids and Phenolics

Total flavonoid concentration of root extracts' is often assessed using a colorimetric aluminum chloride technique that has been previously reported by researchers [66]. The results were expressed as milligrams of quercetin equivalents per gram of dry weight of extract (mg QE/g DW) and a standard curve was created using quercetin. Additionally, the Folin-Ciocalteu procedure was used to measure the variations in the total phenolic content of leaf extracts [66]. Gallic acid was used to find an appropriate calibration curve, and the findings were represented as milligrams of gallic acid equivalent per gram dry weight of extract (mg GAE/g DW).

Determination of Change in Radical Scavenging Potential

The ABTS and DPPH assays were used to examine the changes in the free radical scavenging capability of root extracts after plasma treatment as commonly used by previous researchers [67].

Table 4 Estimation of chlorophyll and total carotenoids content in acetone extract

| Extracting solvents | Chlorophyll a | Chlorophyll b | Total carotenoids |
|---------------------|------------------------------|------------------------------|--|
| Acetone | $12.70A_{663} - 2.69A_{645}$ | $22.90A_{645} - 4.68A_{663}$ | $\frac{1000A_{470} - 1.82[\text{Chl a}] - 85.02[\text{Chl b}]}{198}$ |

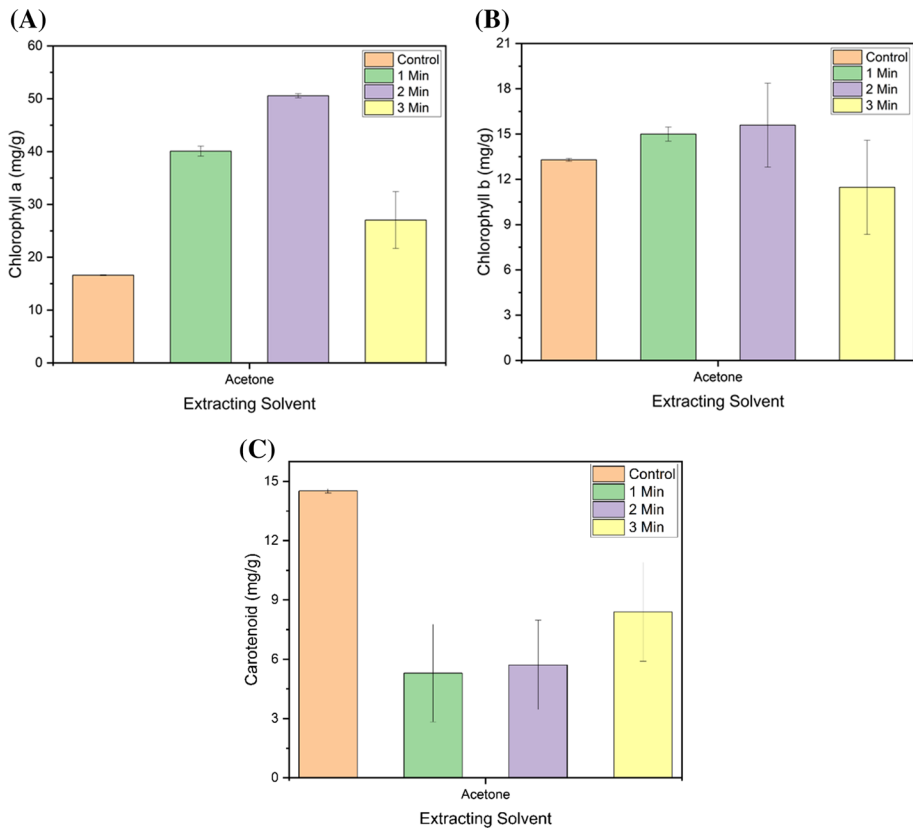


Fig. 14 Estimation of **A** Chlorophyll-a, **B** Chlorophyll-b, and **C** Total carotenoids content before and after LTP treatment

$$\%ABTS/DPPH \text{ Scavenging} = \frac{C - D}{C} \times 100\% \tag{22}$$

where *C* and *D* are the absorbances of the control and test sample, respectively.

Change in Total Flavonoids, Total Phenolic Content, and Radical Scavenging Activities

Flavonoids and phenolics are the major plant secondary metabolites having various health benefits [68]. From last few decades, scientists are interested in identifying novel flavonoids and phenolics and their potential biochemical activities. Plants create chemical called secondary metabolites, which are currently unknown to have any role in photosynthesis, growth, reproduction, or other fundamental activities. The changes in these secondary metabolites indicated the change in environmental conditions and the physiological response of plants [68]. It is already proven that every plant species has some extent of flavonoids and phenolics composition which represents the health and biochemical potential of the plant. To determine the effectiveness of LTP treatment in seeds, the flavonoid and phenolic content of untreated and treated seedlings were compared. Our results show that

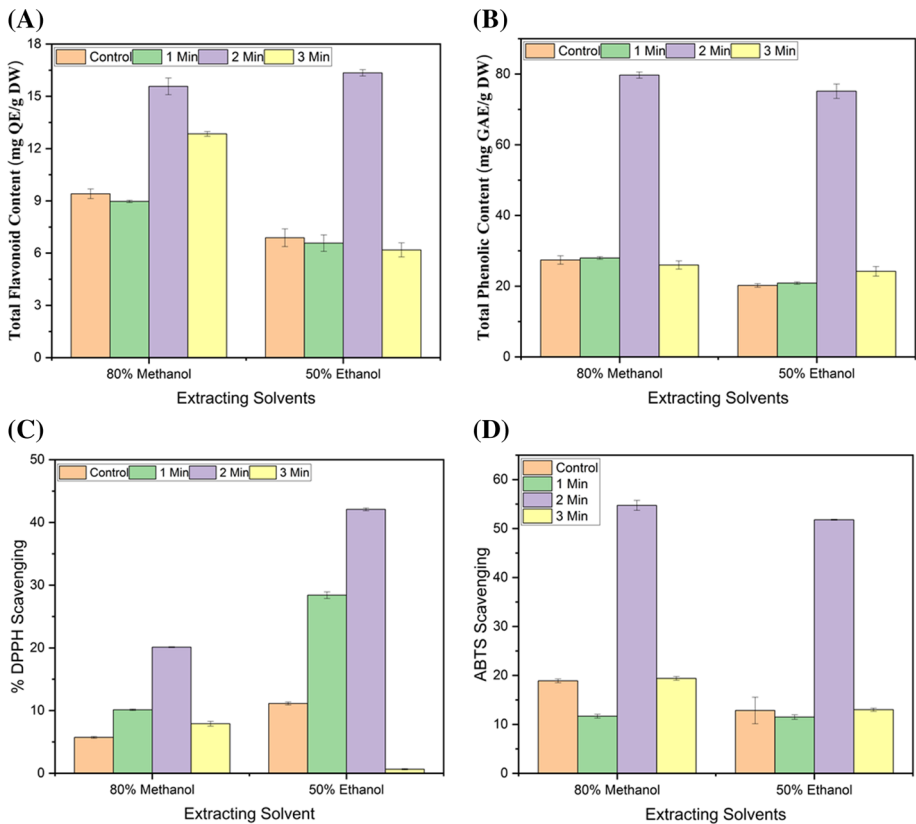


Fig. 15 Estimation of total **A** Flavonoids, **B** Phenolic content, change in **C** DPPH and **D** ABTS scavenging potential, in untreated and treated seedlings extract

the 2 min of plasma treatment boost both flavonoids and phenolics content in the seedling leaf in both ethanol and methanol extracts [Fig. 15 (A, B)].

Moreover, the excessive production of reactive species might cause various complications in plants and animals. The search for natural antioxidants that can act against reactive species is gaining more attention in modern pharmacological and agricultural fields. The radical scavenging potential of plants can be considered as an indicator of the good health of plants and the presence of diverse secondary metabolites within them [69]. So, we attempted to compare the radical scavenging capability of root extracts of untreated and plasma-treated seedlings by both ethanol and methanol extraction. The results showed that plant extracts had a greater ability to scavenge both DPPH and ABTS free radicals when treated for 1 and 2 min and decreased remarkably in 3 min of treatment time [Fig. 15 (C, D)]. This might be due to excessive production of reactive species by plasma after 2 min of treatment.

Analysis of Change in Physical Properties of Water

For the preliminary analysis of activation of seed through plasma treatment; water was analyzed by soaking the untreated and treated seeds into deionized water for 24 h and

Table 5 Change in the physical properties of deionized water by the introduction of plasma- treated seeds

| Parameters | Deionized water | Control | 1-min treatment | 2-min treatment | 3-min treatment |
|-------------------------------|-----------------|---------|-----------------|-----------------|-----------------|
| pH | 7.20 | 7.17 | 6.84 | 6.76 | 6.72 |
| Dissolved oxygen (ppm) | 7.00 | 5.00 | 4.20 | 4.60 | 6.00 |
| Total dissolved solids (ppm) | 0.00 | 28.30 | 49.30 | 37.00 | 34.40 |
| Electrical conductivity (ppm) | 0.00 | 43.50 | 74.50 | 55.00 | 53.00 |
| Turbidity (NTU) | 0.00 | 12.27 | 21.59 | 17.10 | 13.79 |

measuring the change in physical properties of water. The changes in pH, electrical conductivity, total dissolved solids, turbidity, and total dissolved oxygen were analyzed by a standard multi-parameter probe (Lutron, WA-2015). The screening of the effects of plasma on seeds can be characterized and confirmed by analyzing the effects of treated seeds on the physicochemical properties of water. Table 5 revealed the change in physical parameters of analyzed deionized water.

From Table 5, optimum results were observed in 1 min of plasma treatment. Other ions and dissolved compounds were freed during plasma etching, as revealed by electrical conductivity tests, and the wettability of the seeds was improved, as indicated by a reduction in the apparent water contact angle, resulting in increased water imbibition.

Conclusions

The pace at which seeds germinate is a measure of how well a crop may be produced overall. Our research showed that germination parameters, vigor index, and seedlings length were significantly enhanced by LTP treatment. LTP exposure changes the seed surface in a way that improves water absorption by eliminating the topmost layers or porating the surface to improve water contact. SEM images show that the seeds' surfaces become much rougher over time compared to the control. The change in water contact angle on the treated seed can be linked to surface roughness caused by treatment and the attachment/detachment of a polar functional groups of the seed coat. Furthermore, FTIR analysis revealed that the enhancement of the hydrophilic properties of the seeds after the plasma treatment is plausibly related to the damaging of the hydrophobic moieties on the surface of the seeds. Also, LTP treatment resulted in significantly higher levels of Chlorophyll-a in the leaves, but there was no discernible improvement in Chlorophyll-b and total carotenoid content. Our findings showed that two minutes plasma treated seeds had a higher concentration of flavonoids and phenolic content. Plant extracts had a greater ability to scavenge both DPPH and ABTS free radicals in 1 and 2 min in LTP-exposed seeds and decreased remarkably in 3 min of LTP-exposed seeds. Utilizing this technology as a standard procedure for seed treatment before sowing might assist in reducing the consumption of agrochemicals, improving crop productivity without compromising environmental degradation. All the results of our study suggest that early seed sprouting and radish growth can be successfully enhanced by LTP treatment. In our view, field tests should be carried out immediately to observe the impacts of LTP-treated seeds and make them applicable to society, rather than being limited to the lab.

Acknowledgements The authors would want to express gratitude to Prof. Dr. Eun. Ha. Choi (Kwangwoon University, Korea), Prof. Dr. Milan Simek (Institute of Plasma Physics, The Czech Academy of Sciences), Dr. Johannes Gruenwald (Gruenwald Laboratories, Austria), Dr. Duc Ba Nguyen (Jeju National University, Korea), Prof. Jacek Tyczkowski (Lodz University of Technology: Łódź, Poland), Dr. Bhagirath Ghimire (Lancaster University, England), “the Department of Molecular Engineering, Faculty of Process and Environmental Engineering, Lodz University of Technology, Poland” and to “the Nepal Academy of Science and Technology (NAST)”, “Nepal Agricultural Research Council (NARC)”, for their kind support.

Author's Contribution RPG: laboratory work, data collection, analysis, and authorization, as well as the preparation, revision, and editing of the paper. SPP, HBB, BS: Laboratory work, data gathering BPP, DPS: supervision of the task, data verification, MF, HKP: supervision of task, FTIR analysis, and editing of the paper.

Funding The “Ph.D. fellowship award 2076/2077 from the Nepal Academy of Science and Technology (NAST)”, “Kathmandu University-Integrated Rural Development Project (KU-IRDP) /Nepal Technology Innovation Center Project (KU-IRDP/NTIC)”, and “the PROM Programme -International Scholarship Exchange of Ph.D. Candidates and Academic Staff (Lodz University of Technology)” contributed some funding to this study.

Data Availability A reasonable request to the corresponding authors may permit the data used to support the results of this study.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

1. Banihani SA (2017) Radish (*Raphanus sativus*) and diabetes. *Nutrients* 9:1014
2. Shukla S, Chatterji S, Mehta S et al (2011) Antidiabetic effect of *Raphanus sativus* root juice. *Pharm Biol* 49:32–37. <https://doi.org/10.3109/13880209.2010.493178>
3. Il JS, Lee S, Choi BK et al (2010) Methylisogermaullone isolated from radish roots stimulates small bowel motility via activation of acetylcholinergic receptors. *J Pharm Pharmacol* 57:1653–1659. <https://doi.org/10.1211/jpp.57.12.0016>
4. Lamichhane P, Veerana M, Lim JS, et al (2021) Low-Temperature Plasma-Assisted Nitrogen Fixation for Corn Plant Growth and Development. *mdpi.com*. doi: <https://doi.org/10.3390/ijms22105360>
5. Chalise D, Kumar L, Kristiansen P (2019) Land Degradation by Soil Erosion in Nepal: A Review. *mdpi.com*. doi: <https://doi.org/10.3390/soilsystems3010012>
6. Ling L, Jiafeng J, Jiangang L, et al Effects of cold plasma treatment on seed germination and seedling growth of soybean. *nature.com*
7. Ecotoxicology WE-, 1998 undefined Effects of heavy metals in plants at the cellular and organismic level. *research.vu.nl*
8. Los A, Ziuzina D, Boehm D et al (2019) Investigation of mechanisms involved in germination enhancement of wheat (*Triticum aestivum*) by cold plasma: effects on seed surface chemistry and characteristics. *Plasma Process Polym*. <https://doi.org/10.1002/ppap.201800148>
9. Ito M, Ohta T, Hori M (2012) Plasma agriculture. *J Korean Phys Soc* 60:937–943. <https://doi.org/10.3938/jkps.60.937>
10. Šerá B, Špatenka P, Šerý M et al (2010) Influence of plasma treatment on wheat and oat germination and early growth. *IEEE Trans Plasma Sci* 38:2963–2968. <https://doi.org/10.1109/TPS.2010.2060728>
11. Surowsky B, Fischer A, Schlueter O, Knorr D (2013) Cold plasma effects on enzyme activity in a model food system. *Innov Food Sci Emerg Technol* 19:146–152. <https://doi.org/10.1016/j.ifset.2013.04.002>

12. Meiqiang Y, Mingjing H, MB-PS and, 2005 undefined Stimulating effects of seed treatment by magnetized plasma on tomato growth and yield. *iopscience.iop.org*
13. Adamovich I, Baalrud SD, Bogaerts A et al (2017) The 2017 plasma roadmap: low temperature plasma science and technology. *J Phys D Appl Phys* 50:323001
14. Karmakar S, Billah M, Hasan M et al (2021) Impact of LFGD (Ar+O₂) plasma on seed surface, germination, plant growth, productivity and nutritional composition of maize (*Zea mays* L). *Heliyon* 7:06458. <https://doi.org/10.1016/j.heliyon.2021.e06458>
15. Masood A, Auliya RZ, Ahmed N, et al (2021) Effect of H₂ O-Based Low-Pressure Plasma (LPP) Treatment on the Germination of Bambara Groundnut Seeds Assessing public benefit and risk perception of nanotechnology for sustainable development View project Effect of H₂ O-Based Low-Pressure Plasma (LPP) Treatment on the Germination of Bambara Groundnut Seeds. *mdpi.com*. doi: <https://doi.org/10.3390/agronomy11020338>
16. Waskow A, Howling A, Furno I (2021) Mechanisms of plasma-seed treatments as a potential seed processing technology. *Front Phys* 9:617345
17. Weltmann KD, Kolb JF, Holub M et al (2019) The future for plasma science and technology. *Plasma Process Polym* 16:1–29. <https://doi.org/10.1002/ppap.201800118>
18. Ivankov A, Mildaziene V, Degutyte-Fomins L, et al Cold plasma treatment stimulates germination due to rapid changes induced in the content of radish seed phytohormones. *researchgate.net*. doi: <https://doi.org/10.13140/RG.2.2.13138.07364>
19. Tonmitr N, Higa A, Applied AY-JJ of, 2022 undefined (2022) Effect of LF-microwave hybrid plasma treatment on radish seed germination and sprout growth. *Jpn J Appl Phys*
20. Guragain RP, Baniya HB, Dhungana S et al (2021) Effect of plasma treatment on the seed germination and seedling growth of radish (*Raphanus sativus*). *Plasma Sci Technol* 24:015502. <https://doi.org/10.1088/2058-6272/AC3476>
21. Attri P, Ishikawa K, Okumura T et al (2021) (2021) Impact of seed color and storage time on the radish seed germination and sprout growth in plasma agriculture. *Sci Reports* 11:1–10. <https://doi.org/10.1038/s41598-021-81175-x>
22. Gott RP, Engeling KW, Johnson CM et al (2022) Plasma sanitization of cherry belle radish seeds for Space agricultural applications. *Plasma Res Express* 4:025001. <https://doi.org/10.1088/2516-1067/AC64EE>
23. Mildaziene V, Ivankov A, Sera B, Baniulis D (2022) Biochemical and physiological plant processes affected by seed treatment with non-thermal plasma. *Plants* 11:856. <https://doi.org/10.3390/PLANT11070856>
24. Dahal BM, Raut N, Gurung S et al (2018) Changing trends in cultivation practices and adoption of climate adaptive farming in eastern Nepal. *Sustain Agric Res*. <https://doi.org/10.5539/sar.v7n3p52>
25. Pyakuryal B, Roy D, Thapa YB (2010) Trade liberalization and food security in Nepal. *Food Policy* 35:20–31. <https://doi.org/10.1016/j.foodpol.2009.09.001>
26. Pérez-Pizá MC, Prevosto L, Grijalba PE et al (2019) Improvement of growth and yield of soybean plants through the application of non-thermal plasmas to seeds with different health status. *Heliyon*. <https://doi.org/10.1016/j.heliyon.2019.e01495>
27. Ranal MA, de Santana DG, Ferreira WR, Mendes-Rodrigues C (2009) Calculating germination measurements and organizing spreadsheets. *Rev Bras Botânica* 32:849–855. <https://doi.org/10.1590/s0100-84042009000400022>
28. Orchard T (1977) Estimating the parameters of plant seedling emergence. *Seed Sci Technol* 5:61–69
29. Ranal MA, De Santana DG (2006) How and why to measure the germination process? *Rev Bras Bot* 29:1–11. <https://doi.org/10.1590/S0100-84042006000100002>
30. Dastanpoor N, Fahimi H, Shariati M et al (2013) Effects of hydropriming on seed germination and seedling growth in sage (*Salvia officinalis* L.). *African J Biotechnol* 12:1223–1228. <https://doi.org/10.5897/AJB12.1941>
31. Jones KW, Sanders DC (1987) The influence of soaking pepper seed in water or potassium salt solutions on germination at three temperatures. *J Seed Technol* 11:97–102
32. Opoku JA, Amisshah JN, Essilfie ME, Norman JC (2018) Effect of pre-sowing treatments on seed germination and seedling growth of silver butterfly tree (*Bauhinia rufescens*). *Curr Agric Res J* 6:344–354. <https://doi.org/10.12944/carj.6.3.13>
33. Czabator FJ (1962) Germination value: an index combining speed and completeness of pine seed germination. *For Sci* 8:386–396
34. Nersisyan G, Graham WG (2004) Characterization of a dielectric barrier discharge operating in an open reactor with flowing helium. *Plasma Sources Sci Technol* 13:582–587. <https://doi.org/10.1088/0963-0252/13/4/005>

35. Manley TC (1943) The electric characteristics of the ozonator discharge. *Trans Electrochem Soc* 16:450–460
36. Tao X, Lu R, Li H (2012) Electrical characteristics of dielectric-barrier discharges in atmospheric pressure air using a power-frequency voltage source. *Plasma Sci Technol* 14:723–727. <https://doi.org/10.1088/1009-0630/14/8/08>
37. Wagnera H-E, Brandenburga R, Kozlovb KV et al (2003) The barrier discharge: basic properties and applications to surface treatment. *Vacuum* 71:417–436
38. Sarani A, Nikiforov AY, Leys C (2010) Atmospheric pressure plasma jet in Ar and Ar/H₂O mixtures: optical emission spectroscopy and temperature measurements. *Phys Plasmas* 17:063504. <https://doi.org/10.1063/1.3439685>
39. Kim JH, Choi YH, Hwang YS (2006) Electron density and temperature measurement method by using emission spectroscopy in atmospheric pressure nonequilibrium nitrogen plasmas. *Phys Plasmas*. <https://doi.org/10.1063/1.2338282>
40. Ohno N, Razzak MA, Ukai H et al (2006) Validity of electron temperature measurement by using boltzmann plot method in radio frequency inductive discharge in the atmospheric pressure range. *Plasma Fusion Res* 1:028–028. <https://doi.org/10.1585/pfr.1.028>
41. Najarian ML, Chinni RC (2013) Temperature and electron density determination on Laser-Induced Breakdown Spectroscopy (LIBS) plasmas: a physical chemistry experiment. *J Chem Educ* 90:244–247. <https://doi.org/10.1021/ed3003385>
42. Kramida A, Ralchenko Y, Reader J, Team NA (2020) NIST Atomic Spectra Database [Online]. 8:96–102
43. Jiang J, He X, Li L et al (2014) Effect of cold plasma treatment on seed germination and growth of wheat. *Plasma Sci Technol* 16:54–58. <https://doi.org/10.1088/1009-0630/16/1/12>
44. Talská R, Machalová J, Smýkal P, Hron K (2020) A comparison of seed germination coefficients using functional regression. *Appl Plant Sci* 8:e11366. <https://doi.org/10.1002/aps3.11366>
45. Barba-Espín G, Diaz-Vivancos P, Job D et al (2011) Understanding the role of H₂O₂ during pea seed germination: a combined proteomic and hormone profiling approach. *Plant, Cell Environ* 34:1907–1919. <https://doi.org/10.1111/j.1365-3040.2011.02386.x>
46. Li Z, Xu J, Gao Y et al (2017) The synergistic priming effect of exogenous salicylic acid and H₂O₂ on chilling tolerance enhancement during maize (*Zea mays* L.) seed germination. *Front Plant Sci* 8:1153. <https://doi.org/10.3389/fpls.2017.01153>
47. Sasaki K, Kishitani S, Abe F, Sato T (2005) Promotion of seedling growth of seeds of rice (*Oryza sativa* L. cv. Hitomebore) by treatment with H₂O₂ before sowing. *Plant Prod Sci* 8:509–514. <https://doi.org/10.1626/pps.8.509>
48. Cembrowska-Lech D, Koprowski M, Kepczyński J (2015) Germination induction of dormant *Avena fatua* caryopses by KAR1 and GA3 involving the control of reactive oxygen species (H₂O₂ and O₂⁻) and enzymatic antioxidants (superoxide dismutase and catalase) both in the embryo and the aleurone layers. *J Plant Physiol* 176:169–179. <https://doi.org/10.1016/j.jplph.2014.11.010>
49. Molina R, Lalueza A, López-Santos C et al (2021) Physicochemical surface analysis and germination at different irrigation conditions of DBD plasma-treated wheat seeds. *Plasma Process Polym*. <https://doi.org/10.1002/ppap.202000086>
50. Dawood N (2020) Effect of RF plasma on *Moringa* seeds germination and growth. *J Taibah Univ Sci* 14:279–284. <https://doi.org/10.1080/16583655.2020.1713570>
51. Zahoranová A, Henselová M, Hudcová D et al (2016) Effect of cold atmospheric pressure plasma on the wheat seedlings vigor and on the inactivation of microorganisms on the seeds surface. *Plasma Chem Plasma Process* 36:397–414. <https://doi.org/10.1007/s11090-015-9684-z>
52. Lotfy K, Al-Harbi NA, Abd El-Raheem H (2019) Cold atmospheric pressure nitrogen plasma jet for enhancement germination of wheat seeds. *Plasma Chem Plasma Process* 39:897–912. <https://doi.org/10.1007/s11090-019-09969-6>
53. Ling L, Jiafeng J, Jiangang L et al (2014) (2014) Effects of cold plasma treatment on seed germination and seedling growth of soybean. *Sci Reports* 4(4):1–7. <https://doi.org/10.1038/srep05859>
54. Zhang JJ, Jo JO, Huynh DL et al (2017) Growth-inducing effects of argon plasma on soybean sprouts via the regulation of demethylation levels of energy metabolism-related genes. *Sci Rep* 7:41917. <https://doi.org/10.1038/srep41917>
55. Dobrynin D, Fridman G, Friedman G, Fridman A (2009) Physical and biological mechanisms of direct plasma interaction with living tissue. *New J Phys* 11:115020. <https://doi.org/10.1088/1367-2630/11/11/115020>
56. Bormashenko E, Gryniov R, Bormashenko Y, Drori E (2012) Cold radiofrequency plasma treatment modifies wettability and germination speed of plant seeds. *Sci Rep* 2:1–8. <https://doi.org/10.1038/srep00741>

57. Sadhu S, Thirumdas R, Deshmukh RR, Annappure US (2017) Influence of cold plasma on the enzymatic activity in germinating mung beans (Vigna radiate). *LWT - Food Sci Technol* 78:97–104. <https://doi.org/10.1016/j.lwt.2016.12.026>
58. Iranbakhsh A, Ghoranneviss M, Oraghi Ardebili Z et al (2017) Non-thermal plasma modified growth and physiology in *Triticum aestivum* via generated signaling molecules and UV radiation. *Biol Plant* 61:702–708. <https://doi.org/10.1007/s10535-016-0699-y>
59. Varnagiris S, Vilimaite S, Mikelionyte I et al (2020) The combination of simultaneous plasma treatment with mg nanoparticles deposition technique for better mung bean seeds germination. *Processes* 8:1575. <https://doi.org/10.3390/pr8121575>
60. Fang J, Zhang L, Sutton D et al (2012) Needleless melt-electrospinning of polypropylene nanofibres. *J Nanomater.* <https://doi.org/10.1155/2012/382639>
61. Alshuaib SM, Al-Ghouti MA (2020) Multivariate analysis for FTIR in understanding treatment of used cooking oil using activated carbon prepared from olive stone. *PLoS ONE* 15:e0232997. <https://doi.org/10.1371/JOURNAL.PONE.0232997>
62. Ronzhina DA, Yudina PK (2013) Changes in the chlorophyll and carotenoid contents in the leaves of steppe plants along a latitudinal gradient in South Ural. *Artic Russ J Plant Physiol* 60:856–864. <https://doi.org/10.1134/S1021443713050075>
63. Aadil RM, Roobab U, Sahar A et al (2019) Functionality of Bioactive Nutrients in Beverages. *Nutr Beverages Sci Beverages* 12:237–276. <https://doi.org/10.1016/B978-0-12-816842-4.00007-1>
64. Wellburn AR (1994) The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J Plant Physiol* 144:307–313. [https://doi.org/10.1016/S0176-1617\(11\)81192-2](https://doi.org/10.1016/S0176-1617(11)81192-2)
65. Saberi M, Modarres-Sanavy SAM, Zare R, Ghomi H (2018) Amelioration of photosynthesis and quality of wheat under non-thermal radio frequency plasma treatment. *Sci Rep* 8:1–8. <https://doi.org/10.1038/s41598-018-30200-7>
66. Prasad Pandey B, Prakash Pradhan S, Adhikari K (2020) LC-ESI-QTOF-MS for the profiling of the metabolites and in vitro enzymes inhibition activity of Bryophyllum Pinnatum and Oxalis Corniculata collected from ramechhap district of Nepal. *Chem Biodivers.* <https://doi.org/10.1002/cbdv.202000155>
67. Pandey BP, Pradhan SP, Adhikari K, Nepal S (2020) *Bergenia pacumbis* from Nepal, an astonishing enzymes inhibitor. *BMC Complement Med Ther* 20:198. <https://doi.org/10.1186/s12906-020-02989-2>
68. Miller NJ, Begoña Ruiz-Larrea M (2002) Flavonoids and other plant phenols in the diet: their significance as antioxidants. *J Nutr Environ Med* 12:39–51
69. Ning W, Peng X, Ma L et al (2012) Enhanced secondary metabolites production and antioxidant activity in postharvest *Lonicera japonica* Thunb. in response to UV radiation. *Innov Food Sci Emerg Technol* 13:231–243. <https://doi.org/10.1016/j.ifset.2011.10.005>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Rajesh Prakash Guragain¹ · Hom Bahadur Baniya² · Suman Prakash Pradhan³ · Bishnu Prasad Pandey⁴ · Bikash Shrestha¹ · Maciej Fronczak⁵ · Hanna Kierzkowska-Pawlak⁵ · Deepak Prasad Subedi¹

¹ Department of Physics, Kathmandu University, Dhulikhel, Kavre, Nepal

² Department of Physics, Tri-Chandra Multiple Campus, Tribhuvan University, Ghantaghar, Kathmandu, Nepal

³ Department of Environmental Science and Engineering, Kathmandu University, Dhulikhel, Kavre, Nepal

⁴ Department of Chemical Science and Engineering, Kathmandu University, Dhulikhel, Kavre, Nepal

⁵ Department of Molecular Engineering, Faculty of Process and Environmental Engineering, Lodz University of Technology, 90-924, Lodz, Poland