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Effect of H_2O-Based Low-Pressure Plasma (LPP) Treatment on the Germination of Bambara Groundnut Seeds

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Abstract: In general, seed germination is improved by low-pressure plasma (LPP) treatment using precursors such as air, nitrogen, argon, or water (H_2O). Here, H_2O-based LPP treatment using the optimized parameters of 10 W and 10 s improves the germination of Bambara groundnut seeds by 22%. LPP increases the wettability and roughness of the seed hilum while oxidizing the surface with carboxyl and amine groups. In this H_2O-based treatment of Bambara groundnut seeds, combinatory etching and chemical modification facilitated the imbibition process and increased the germination percentage. The success of this method has the potential to be scaled up to solve food security with seeds otherwise facing germination-related issues.

Keywords: Bambara groundnut; germination; low-pressure plasma (LPP); plasma treatment; wettability

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1. Introduction

Rapid population growth means the world is facing food shortage problems, and at present only 41 crops account for over 90% of all food calories grown in the world [1]. This reliance on a small number of crops poses a food supply risk due to the confluence of this small pool of species, the genetic base of agriculture, and climate change.

Accordingly, some under-utilized crops are being explored as possible substitutes and complements by various agricultural policy think tanks and research communities. One such species is the Bambara groundnut that grows extensively in sub-Saharan Africa [2,3]. Nutritionally, it is the third most crucial legume species in semi-arid Africa, after the cowpea (*Vigna unguiculata*) and groundnut (*Arachis hypogaea*) [4]. Specifically, Bambara groundnut contains 18–24% protein with high methionine and lysine contents, 4–12% crude oil, 51–70% carbohydrate, 3–12% fiber, and 3–5% ash [5]. As a legume, Bambara groundnut additionally contributes to soil fertility by fixing atmospheric nitrogen and complementing cereal crops both nutritionally and agronomically [5]. Bambara groundnuts are eaten and processed in many ways; they can be used as boiled grains, eaten as a snack, made into flour for subsequent food processing, and used as feed for poultry and livestock [6–8].

Although the Bambara groundnut's merit as a drought-resistant food crop in dry areas has gained attention in recent years, it still lacks a proper seed improvement system [5]. Amongst the various problems related to the Bambara groundnut planting are pathogen attack, low yields, and slow germination [9,10]. Some germination techniques have been utilized to overcome these problems; for example, Bambara groundnut seeds have been exposed to solar heat [11] and also primed before sowing [12], but there is still a need for a cost-efficient, scalable, and environmentally friendly approach that can overcome the problems mentioned earlier.

Low-pressure plasma (LPP) has been suggested as an environmentally friendly method to reduce pathogen infection, improve germination, and thereby increase yield. For example, a 50% increment in safflower germination was observed when the seed surface was modified by low-pressure radio-frequency argon discharge [13]. Another food crop, brown rice, showed an up to 62% increase in germination percentage when treated by low-pressure plasma [14]. Likewise, a significant improvement was observed in the germination of chili and cucumber seeds when treated with air plasma [15], and LPP treatment similarly increased the yields of winter wheat, maize, and narrow-leaved lupine by 2.3%, 1.7%, and 26.8%, respectively [16]. The plasma treatment of tomato seeds also increased their yield to 20.7% relative to untreated seeds [17]. Separately, the dielectric barrier discharge (DBD) treatment of barley seed increased hypocotyl growth from 15% to 110% depending on the plasma treatment duration [18].

Generally, LPP treatment for agriculture applications utilizes water (H_2O), oxygen, nitrogen, and argon as monomers because of their environmental friendliness and long-lasting effect [19–23]. Here, this study used H_2O as a monomer for plasma treatment because, in addition to environmental friendliness and long-lasting treatment, this method provides an etching effect [24]. The etching capability of H_2O plasma on an organic solid is 40 times more efficient than an oxygen plasma [25]. While the positive effect of plasma treatment is evident in reported data, the exact mechanism underlying this enhanced germination and growth is less clear and likely to be dependent on the seed type. Generally, improvements in germination can be attributed to a combination of factors such as hydrophilicity, wettability, and an increase in water absorption [21].

The primary aim of the current work was to investigate the influence of H_2O-based LPP treatment on the Bambara groundnut germination, as vacuum alone has been shown to have mixed results [26–28]. Furthermore, this study aimed to suggest the mechanism responsible, applying various assays such as the measurement of water uptake and electrical conductivity of the liquid used to submerge Bambara groundnut seeds. We also analyzed physico-chemical changes in the H_2O-LPP-treated Bambara groundnut seeds in terms of the water contact angle (WCA), X-ray photoelectron spectroscopy (XPS), and field emission scanning electron microscopy (FESEM).

2. Materials and Methods

Bambara groundnut (*Vigna subterranean*), which belongs to the Fabaceae family, was provided by Crops For Future, Malaysia. This study used Bambara groundnut seeds without visible signs of infection and defects.

H_2O-low-pressure plasma treatment was performed in a custom-built reactor [29,30] consisting of an upper U-shaped copper electrode and a bottom circular copper electrode (Figure 1). The plasma was generated with an impedance matching that of a radio-frequency generator (RF-3-XIII) operating at 13.56 MHz. Twenty Bambara groundnut seeds were placed on the bottom electrode for each replicate, and then the pressure was pumped down to ~6.6 Pa to start the experiment. The precursor, H_2O, was then fed through a needle valve into the reactor. A CVM211 stinger vacuum gauge monitored the pressure inside the plasma chamber. Upon achieving a stable pressure of ~40 Pa, the Bambara groundnut seeds were subjected to H_2O plasma treatment at two different power levels, 10 and 30 W, for durations of 10, 20, and 30 s. The pressure increased to 46 Pa during the plasma ignition.

All the germination tests complied with the rules of the International Seed Testing Association (ISTA). Several factors influence seed germination—namely, substrate type (e.g., sand, soil, or filter paper), seed positioning (e.g., between filter papers or on top of filter paper), lighting regime (e.g., natural or artificial), and temperature regime (e.g., constant or changing).

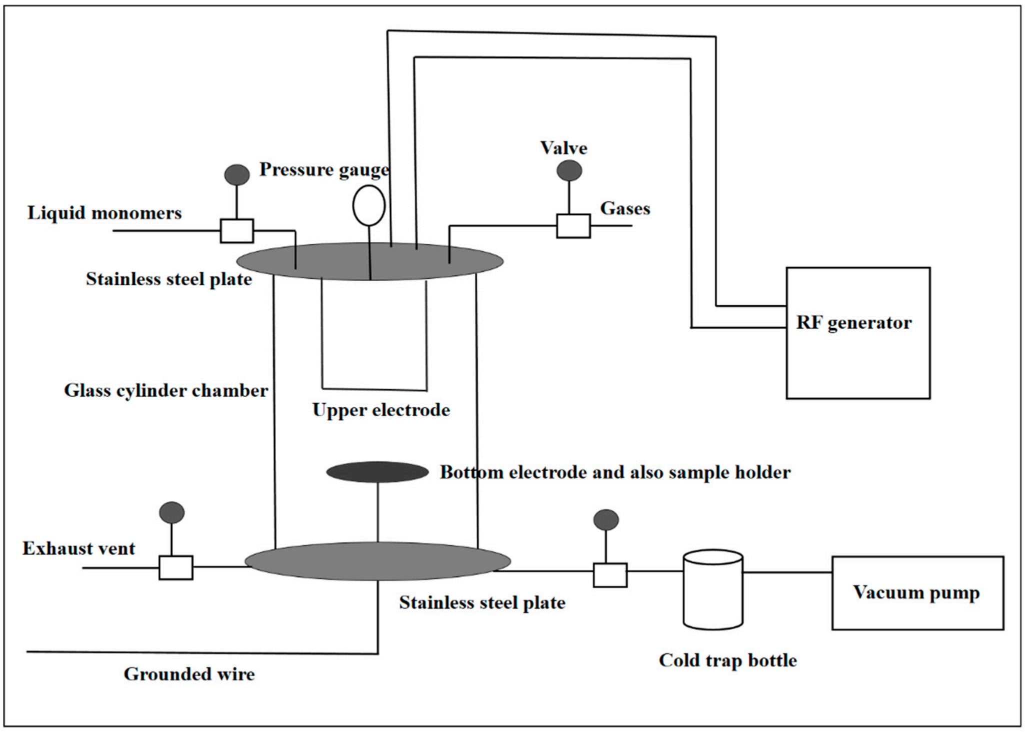

Figure 1. Schematic diagram of the experimental setup used in this study.

In this germination study, Bambara groundnut seeds were germinated on trilayer filtration paper in plastic boxes to sustain humidity. Each germination experiment used twenty seeds (weighing ~5 g) and was carried out in five replicates. As per ISTA rules, our seeds were incubated at 25 °C for eight days with the relative humidity (RH) maintained between 60% and 65%. A total of 10 to 15 mL of de-ionized (DI) water was added every 24 h to maintain moisture for germination. Germinated seeds were counted on the 5th day (known as germination potential); another count was carried out on the 8th day after sowing (known as germination percentage (%)) [31], similarly using Equation (1), but with a different germination day. A seed was considered germinated if its radicle length was more than 2 mm [32]. In addition, the root and shoot lengths of each germinated seed were measured on the 11th day. Germination % and seed vigor index (SVI) were calculated using Equations (1) [21] and (2) [33], respectively:

$$G\% = \frac{n \times 100\%}{N}, \tag{1}$$

$$\mathrm{SVI} = G\% \times \mathrm{S}, \tag{2}$$

where n is the number of seeds germinated on a measured day, N is the total number of seeds, and S is the length of the shoot. The germination results were statistically analyzed with the Minitab 17 software. Means were calculated and compared using one-way ANOVA with correction for multiple comparisons per Dunnett's test. Means were considered significantly different when $p < 0.05$.

Apparent water contact angles (WCAs) were measured using a high-resolution camera with a macro-lens setup. Measurements were repeated five times with droplets of ~5 µL DI water on the Bambara groundnut seed hilum. ImageJ (ver. 1.52a; (national institutes of health and labortary for optical and computational instrumentation, Wisconsin, US) Contact Angle plugin) was used to determine the WCAs from their respective images [34]. WCA changes were also measured at periodic intervals for up to 60 days to check for any signs of hydrophobic recovery by the plasma-treated seeds.

Initial WCA measurements were made with the Bambara groundnut seeds exposed to UV radiation produced by a DENG UV lamp (wavelength of 265 nm) for 30 s to determine the influence of UV per se on the WCA measurement. Subsequent measurements were taken directly after LPP treatments.

For water uptake measurements, ~5 g of untreated and plasma-treated seeds were selected and weighed on an electronic balance to a precision of 0.1 mg. The seeds were then immersed in 40 mL of DI water for 24 h at room temperature before having their weights measured again.

The electrical conductivity (EC) of DI water indicates the degree to which ions (electrolyte) leak from the protoplast space or seed apoplast. First, ~5 g of Bambara groundnut seeds without visible damage were selected for this EC test. The untreated and LPP-treated seeds were weighed to a precision of 0.1 mg and immersed in 40 mL of DI water for 24 h. Then, the total dissolved solids (TDS) was determined and an EC holdmeter was used to measure the conductivity of the immersing DI water.

Surface morphology studies of the Bambara groundnut seed hilum were carried out with a Hitachi SU8230 field emission scanning electron microscope (FESEM) (Hitachi high tech corporation, Tokyo Japan) operating in back-scattered mode at an accelerating voltage of 10 kV. Another FESEM (Carl Zeiss, model: Zeiss Supra 55VP Oberkochen, Germany) operating at a similar accelerating voltage and secondary electron mode was also used to image these seeds. All the seeds were gold-coated before imaging. The surface chemical properties were additionally analyzed with X-ray photoelectron spectroscopy (XPS)—i.e., by an ULVAC Quantera II instrument, (Hagisono, Japan) operating with an Al Kα monochromatic source (1486.6 eV). High-resolution spectra of the C1s and N1s regions were component-fitted with the CasaXPS software (ver. 2.3.22PR1.0). The curve-fitting procedure adopted a Shirley-type background with Gaussian–Lorentzian (30% Lorentzian component) as the spectral line shape. During curve fitting, the XPS spectra were calibrated with the binding energy (BE) of the "neutral" C peak (i.e., C-C and C-H components) as 285.0 eV.

Optical emission spectroscopy (OES) was additionally conducted with an Ocean Optics USB2000 (Dunedin, Florida, USA) to identify the plasma species generated during plasma treatment. The emitted radiation was collected via optical fiber (727-733-2447) located at a distance of 3 cm from the plasma chamber.

3. Results and Discussion

3.1. Seed Germination

Generally, plasma treatment modifies plant seeds by etching and chemically modifying the surfaces to encourage the diffusion of nutrients and water, thus inducing germination and growth. Figure 2 shows that the H_2O LPP-treatment of Bambara groundnut seeds significantly improved the germination percentage (%), germination potential, root length, shoot length, and seed vigor index (SVI).

Figure 2 demonstrates that the germination potentials for all H_2O plasma-treated Bambara groundnut seeds were also significantly higher than the control except at 10 W 30 s, a treatment which showed an equivalent potential to the control seeds. In terms of germination %, the highest increment (22%) was observed under H_2O-LPP treatment at 10 W for 10 s. Bambara groundnut seeds treated with H_2O plasma under the conditions of 10 W 20 s, 10 W 30 s, and 30 W 10 s showed respective germination percentage increments of 15%, 3%, and 7%.

The total root lengths for all H_2O-LPP-treated Bambara groundnut seeds were significantly higher than for control seeds, with the specific increment varying from 37% to 45% (note: root length was measured on the 11th day, shown in Figure 3; root length on day 6 is also presented in Figure 3). Similarly, a 10% to 20% increase in shoot length was observed for all H_2O-LPP-treated seeds relative to the untreated seeds. The seed vigor index (SVI) was likewise significantly higher for all H_2O-LPP-treated seeds than for the untreated seeds; the highest SVI was observed for with the 10 W 10 s treatment (please refer to Supplementary S1 for the full data in table format).

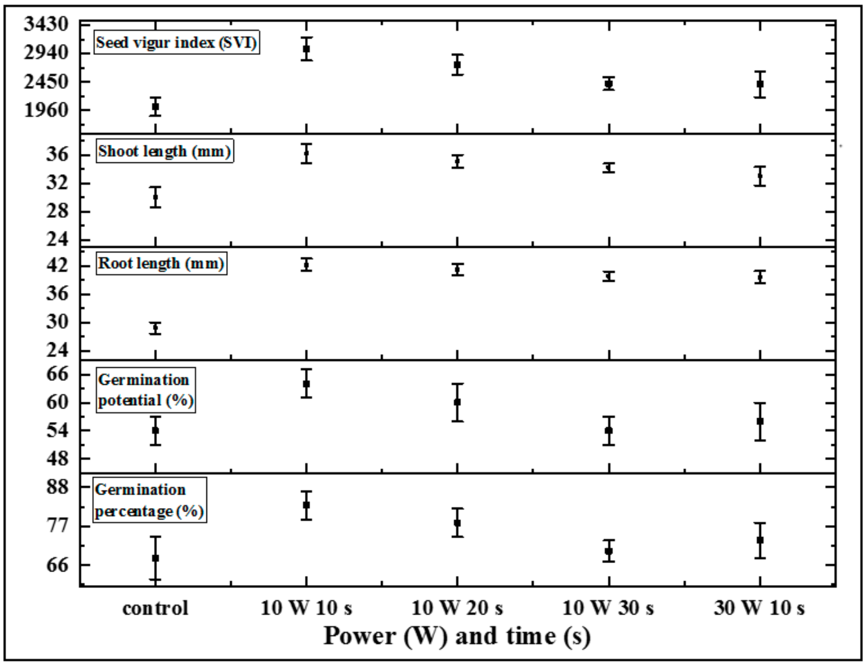

Figure 2. Germination potential (%, first count, day 5), germination percentage (%, final count, day 8), root length (mm), shoot length (mm), and seed vigor index (SVI) of Bambara groundnut seeds (H_2O-plasma-treated and control). Experiments were performed in five replicates, n = 5 (note: 20 seeds weighing ~5 g were used per replicate). Error bars refer to the standard deviations of the five replicates.

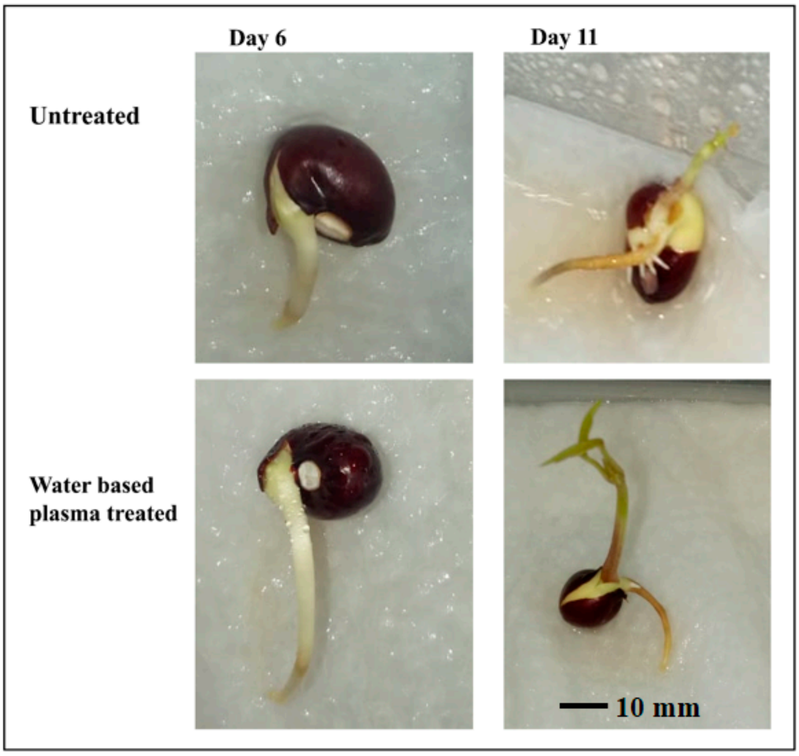

Figure 3. Photographs of the H_2O-LPP-treated and untreated Bambara groundnut seeds 6 and 11 days after germination.

3.2. *Water Contact Angle (WCA)*

The WCA characterizes the wettability and hence permeability of the seed surface. As a hydrophilic surface absorbs more water by which to trigger germination than a hydrophobic surface does, an improvement in hydrophilicity, signified by reducing WCA on the seed surface, is critical in improving seed germination. Although this research was scoped to study the influence of H_2O-LPP treatment on groundnut seeds, we also checked the influence of vacuum on seed wettability and WCA for greater context. Bambara groundnut seeds were evacuated at a similar vacuum level and for a similar duration as achieved with the H_2O-LPP treatment, after which WCAs were measured; no significant difference was observed, indicating that vacuum exposure by itself does not influence the wetting properties of seeds.

While vacuum alone did not influence the seed WCA, H_2O-LPP treatment clearly and significantly reduced the WCA when compared to untreated seeds (Figure 4). A similar reduction in WCA has been reported for wheat seeds exposed to air plasma treatment for 15 s; there, the WCA values of treated seeds registered 0° (complete wetting), improved from 115° (untreated) [35]. While that study and the present work differ in the monomers used and the degree of wettability achieved, both demonstrate a significant reduction in WCA. In the current study, H_2O plasma treatment with more aggressive parameters (e.g., 10 W 30 s and 30 W 10 s) did not necessarily produce the lowest possible WCAs, but rather all treatments yielded quite similar WCAs amongst themselves (Figure 4). Likewise, different plasma treatment durations (from 10 to 30 s) also did not significantly impact the WCA, as the discharge power of 10 W was evidently sufficient to exceed the wettability threshold and maintain the WCA based on the physical and chemical changes observed with FESEM and XPS, discussed below. This discharge power probably signified the threshold WCA, beyond which additional physical-chemical changes do not affect the mechanisms associated with the germination process.

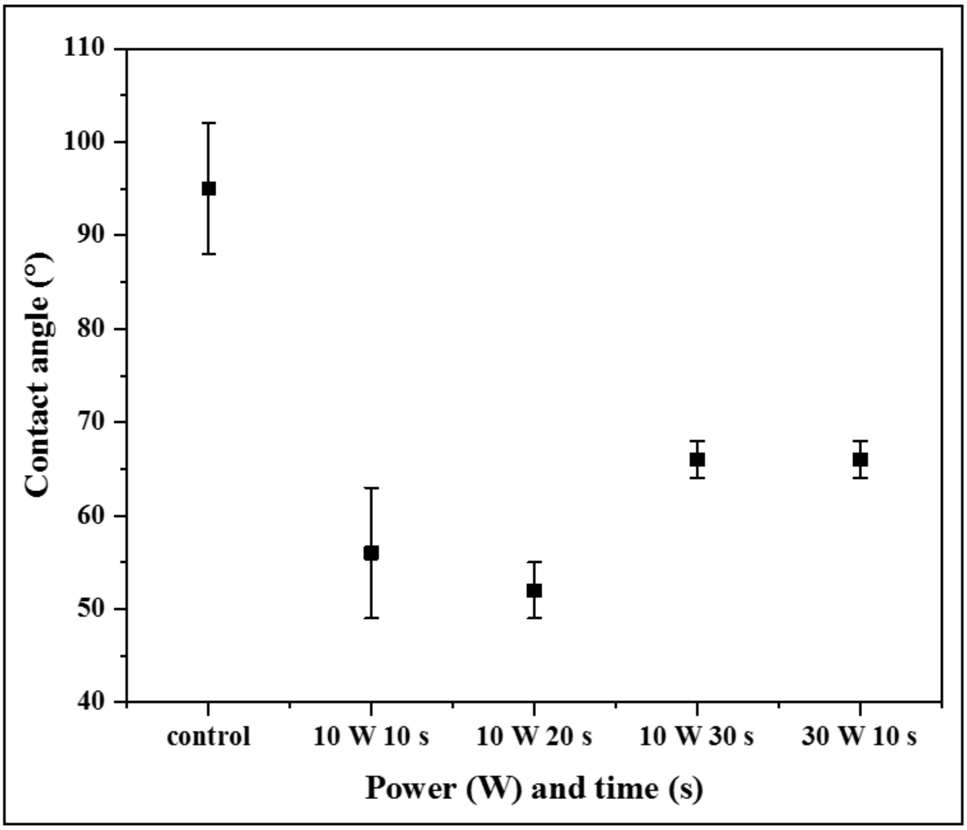

Figure 4. Water contact angle measurements of H_2O-LPP-treated and untreated Bambara groundnut seeds. Experiments were performed in five replicates, $n = 5$, with five seeds per replicate. Error bar refers to standard deviations for the five replicates. All plasma-treated groups are significantly different from the control group ($p < 0.05$).

Although plasma treatment increases the hydrophilicity of synthetic or plasma polymers, those materials also suffer from hydrophobic recovery—that is, the induced hydrophilicity reduces with time, ultimately reverting to the original hydrophobic state [36,37]. Such recovery was not observed in the present study for plasma-treated seeds over 60 days of storage in an ambient atmosphere. A similar lack of hydrophobic recovery has also been reported for other seeds such as lentils, beans, and wheat [35]. The hydrophobic recovery mechanism depends on the processing, materials, and enthalpy-related factors such as precursor type, substrate, treatment conditions, and aging environment [37,38]. The lack of hydrophobic recovery in this study was coincidental but beneficial because the plasma-treated seeds could be stored for a long duration to be planted later. The mechanism underlying this observation is currently being investigated and will be reported in our subsequent work.

3.3. Effect of UV Radiation

It is well known that the wetting properties of synthetic polymers are improved by UV radiation [39] and that the emission of UV radiation accompanies plasma formation [40]. To check the effect of UV radiation on seed wettability, groundnut seeds were exposed to UV radiation for 30 s (the maximum plasma treatment time). The results (Table 1) revealed no significant differences in the water uptake and WCA values between untreated and UV-treated seeds. Thus, the WCA and water uptake of Bambara groundnut seeds are not affected by UV radiation from plasma treatment, but instead by the ions and other species present in H_2O plasma.

Table 1. Effect of UV radiation on the water contact angle and water uptake of Bambara groundnut seeds.

Seed Type	Water Uptake Per Gram (g)	Water Contact Angle (°)
Untreated seeds	0.6158 ± 0.0604	95 ± 7
UV-treated	0.6102 ± 0.0721	94 ± 6

3.4. Water Uptake Analysis

Seed water uptake capacity is a good indicator of seed imbibition. Generally, the first stage of seed germination involves water absorption or uptake through the seed testa or hilum, which activates the hormone-based transport of nutrients necessary for growth. An increase in water uptake means an increase in the absorption of nutrients [23], and that absorption generates the energy to initiate the germination process.

Figure 5 shows that H_2O-LPP treatment significantly increases the water uptake of Bambara groundnut seeds. For example, when H_2O-LPP treatment was carried out at 10 W for 10 s, the seed water uptake values increased by 27%; in contrast, plasma treatment at 10 W for either 20 or 30 s resulted in slight decreases of 20% and 17%, respectively. Unlike the WCA measurement in Section 3.2, the water uptake increased with the increased discharge power, albeit non-linearly. Namely, the water uptake value for seeds that were plasma-treated at 30 W for 10 s was 36% higher compared to the control seeds.

For seeds with a thick hilum and low permeability, such as Bambara groundnut, H_2O-LPP treatments could etch and improve the surface hydrophilicity and thus enhance the water uptake to stimulate germination [20]. However, increased water uptake does not necessarily correlate linearly to germination enhancement because high plasma doses crack the surface (visible in the SEM micrographs in Section 3.6), thus simultaneously reducing the barrier to water diffusion and damaging the seed [41]. This was most evident in the seeds that were plasma-treated at 30 W for 10 s, which showed the highest water uptake (Figure 5) but achieved a low germination percentage and potential (Figure 2). Instead, the highest germination rate was displayed by the seeds treated at 10 W for 10 s, which demonstrated a similar water uptake as those treated at 10 W for either 20 or 30 s.

Nevertheless, the water uptake of seeds treated at 10 W for 10, 20, and 30 s was significantly higher ($p < 0.05$) after 24 h imbibition than that recorded for untreated seeds.

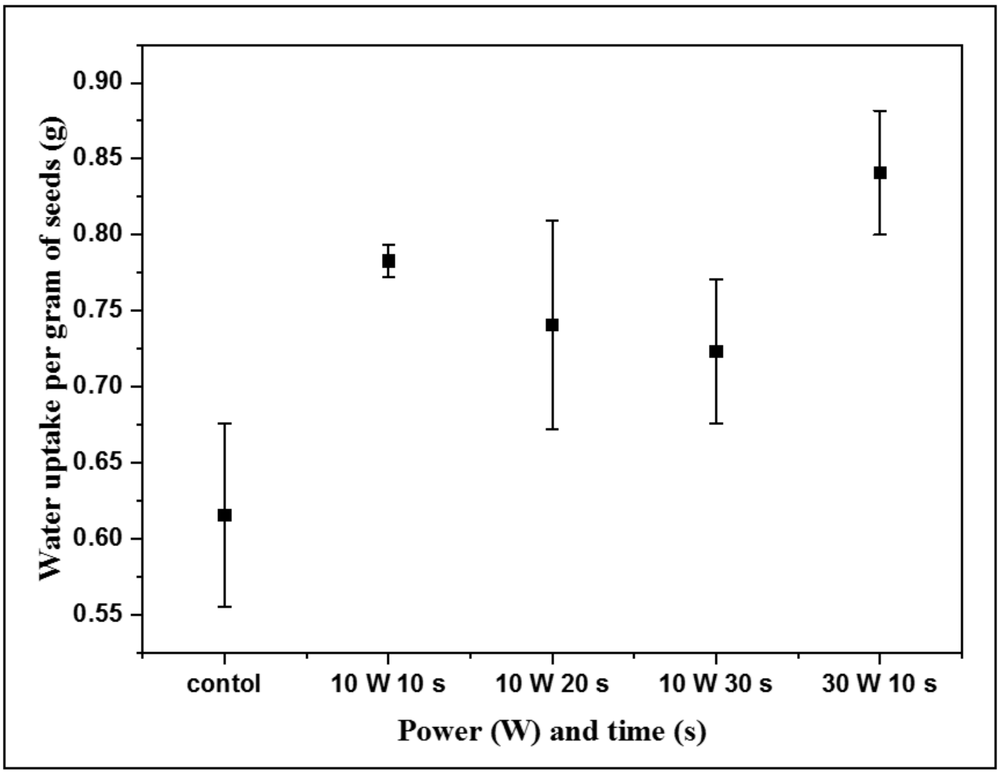

Figure 5. Water uptake of H_2O-LPP treated and untreated Bambara groundnut seeds, measured for ~5 g of Bambara groundnuts before being normalized per unit gram. Experiments were performed in five replicates, $n = 5$ (20 seeds, i.e., ~5 g, were used in each replicate). Error bars refer to the standard deviations of the five replicates.

Although water availability is a critical factor for seed germination, the retention of a water film on the seed surface can reduce the oxygen supply to the seeds and so reduce germination. The water uptake value depends on (1) the seed composition, (2) the permeability of the seed hilum, and (3) water availability. While the Bambara groundnut seed is likely to have relatively uniform composition in the cellulose and lignin region, its surface morphology appeared to be relatively rough, affecting water availability, retention, and absorption on the seed surface. The permeability of the seed hilum could be improved by etching with plasma ions, with plasma treatment at 30 W for 10 s being likely to exceed the critical discharge power needed to create deep etching for water uptake yet also maintain seed viability.

3.5. Electrical Conductivity

When seeds are exposed to excessively intense plasma or chemical treatment, the cell membranes could be weakened, leading to the leaching of intercellular substances such as sugars and electrolytes, the abundance of which can indicate seed vigor. By measuring the electric conductivity of water, ion leakage from the protoplast space of seeds could be investigated and compared between different plasma treatments [42]. As shown in Figure 6, solutions containing H_2O-LPP-treated seeds had a significantly higher electrical conductivity than those containing non-treated seeds ($p < 0.05$); specifically, the increase in solution electrical conductivity was 60%, 51%, 42%, and 74% for seeds plasma-treated at 10 W 10 s, 10 W 20 s, 10 W 30 s, and 30 W 10 s, respectively. This suggests that a greater amount of electrolytes leached out as the treatment intensity increased, but not exposure duration.

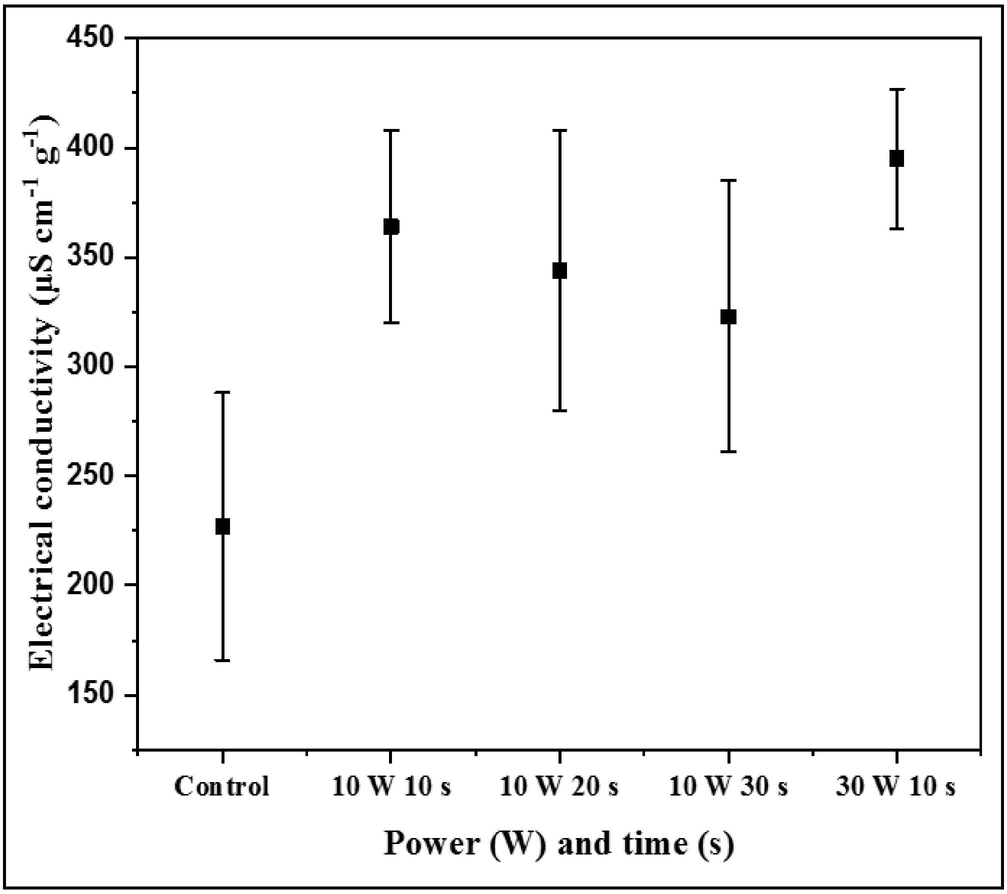

Figure 6. Electrical conductivity (µS cm^{-1} g^{-1}) of solutions containing H_2O-LPP-treated and untreated Bambara groundnut seeds. Experiments were performed in five replicates, $n = 5$ (20 seeds—i.e., ~5 g, were used in each replicate). Error bars refer to the standard deviations of the five replicates.

However, the relationship between the solution's electrical conductivity and germination percentage is not straightforward, as discussed elsewhere. Some researchers claimed that a change in the electrical conductivity of air-plasma-treated seed implies a change in the water permeability and germination rate [42]. Others came to a different conclusion—that high electrical conductivity of the solution soaking soybean seeds suggests a low seed vigor [43]. Still other research has suggested that a high electrical conductivity indicates a high number of dead seeds, leading to a low germination rate [44]. In that vein, an alternative method has been proposed to measure the germination percentage based on electrical conductivity [45]. The high electrical conductivity observed during the aging process was attributed to leakage from degenerated seed membranes.

These different interpretations are each ascribed to different underlying mechanisms, with the involvement of specific ions such as K^+/Na^+ and seed conditions affecting the electrical conductivity test [46]. Due to our pre-selection of unblemished Bambara groundnut seeds, our results were likely to be consistent with those from earlier plasma-treated seeds: the plasma ions etched the seed hilum, allowing ions to diffuse out to the surrounding solution soaking the seeds, while the seeds simultaneously imbibed solution to activate the growth hormone and supply nutrients to support germination and plant growth. That is, H_2O-LPP treatment accelerated the first stage in water imbibition and thereby assisted Bambara groundnut germination.

Besides etching, the plasma ions also oxidized the seed hilum, as shown in the XPS results (Section 3.7). This oxidized seed hilum was ionized, and the associated electrolytes were released into the solution surrounding the seed hilum, increasing the solution's electrical conductivity. Simultaneously, the etching of seeds also allowed the surrounding water to diffuse in and germinate the seeds.

3.6. Surface Morphology

As illustrated in Figure 7a–f, H_2O-LPP treatment changed the seeds morphologically—namely, the hilum became more porous than that of seeds that were not plasma-treated. Water plasma ions are primarily hydroxyl ions, which are highly erosive and capable of etching Bambara groundnut seeds, albeit non-uniformly. It has also been noted that H_2O LPP-treatment is 40× more efficient in etching than oxygen plasma [25]. This further supports the etching effect of H_2O-LPP treatment on the seed hilum and the associated increased water uptake and EC measurements mentioned in Sections 3.4 and 3.5, which accelerated seed germination.

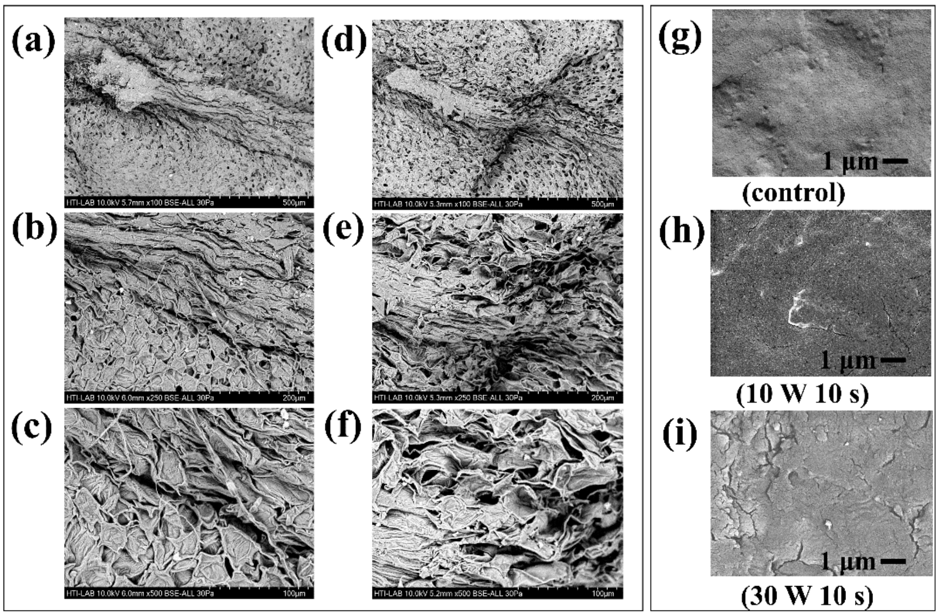

Figure 7. Scanning electron micrographs of (**a**–**c**) untreated and (**d**–**f**) H_2O-plasma-treated Bambara hilum (10 W, 10 s). (**g**–**i**) compares the (**g**) untreated, (**h**) optimized (10 W 10 s), and (**i**) aggressive (30 W 10 s) treatment parameters (scale bars for (**a**–**f**) are 500, 200, and 100 µm respectively. (**g**–**i**) have scale bars of 1 µm.).

Figure 7g–i differentiate the effect of plasma treatment intensity on the surface of Bambara groundnut. Figure 7g shows the absence of visible cracks on untreated Bambara groundnut seeds; in contrast, Figure 7h illustrates how the application of lower-intensity H_2O-LPP treatment (10 W 10 s) slightly cracks Bambara groundnut seeds. These slight cracks increased water uptake, which stimulated germination, as mentioned in Section 3.4. However, a high plasma dosage (30 W, 10 s) cracked the surface, destroyed the barrier to water diffusion, and likely damaged the seeds, as depicted in Figure 7i. The etchability of the seed coating, and by extension the seed type, influences the effectiveness of plasma treatment regardless of whether atmospheric or low-pressure plasma is applied [15,47]. Even seeds of the same variety but with different seed colors respond differently to similar plasma treatments, albeit to a degree only observable under alternative analytical techniques [48].

3.7. X-ray Photoelectron Spectroscopy (XPS)

X-ray photoelectron spectroscopy (XPS) was used to examine the surface chemistry changes and surface composition of LPP-treated Bambara groundnut seeds, specifically in terms of oxidation (O/C) and nitrogen (N/C) content (Table 2). Only untreated seeds and those exposed to H_2O-LPP treatment for 10 s at 10 W were analyzed.

Table 2. Atomic concentrations of the selected elements present in H_2O-LPP-treated and -untreated Bambara groundnut seeds.

Element	C	N	O	N/C	O/C
Control	81.0	4.0	15.0	0.05	0.18
Treated	67.8	5.0	27.2	0.07	0.40

Deconvolutions of high-resolution C1s spectra of Bambara groundnut seeds (Figure 8) showed the presence of four different carbon bonds: C-C/C-H (285 eV), C-O (286.6 eV), C=O (288 eV), and COO (289.3 eV). There was also an evident increase in the H_2O-LPP-treated seeds of moieties related to C-O/C-N (24.4 at%), C=O (10.7 at%), and COO (3.9 at%), formed through oxidation upon exposure to the atmosphere after plasma treatment. In contrast, the corresponding atomic percentages for untreated Bambara groundnut seeds were 14.1 at% for C-O/C-N, 7.3 at% for C=O, and 1.1 at% for COO. The oxidation was similar to that observed for synthetic polymers because of the groundnut lignin-cellulose having similarity with other organic substrates.c

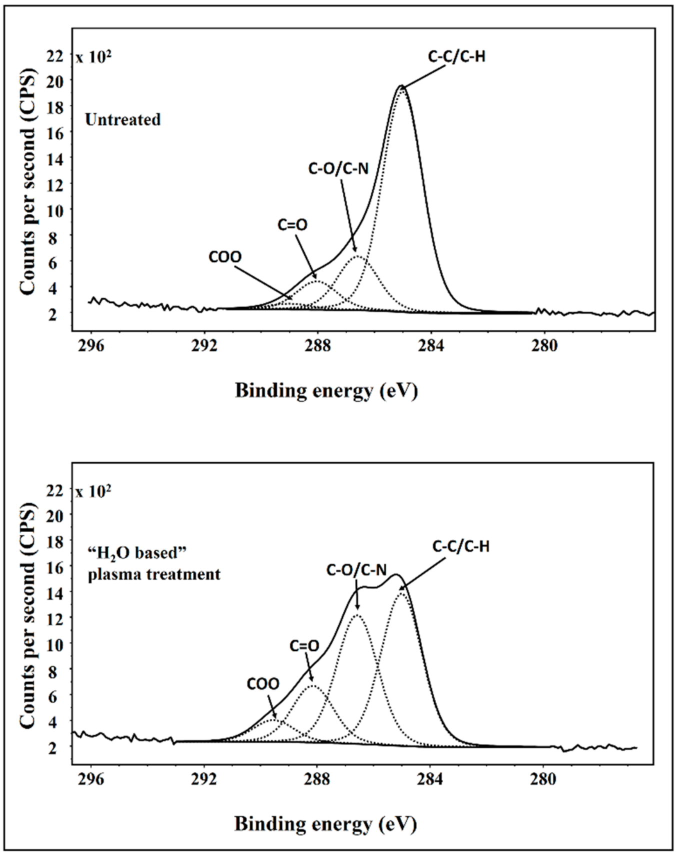

Figure 8. Component fitting of high-resolution C1s spectra of untreated and 10 s H_2O-LPP-treated Bambara groundnut seeds.

Although C-O and C-N are generally separated during component fitting in the literature, this study decided to combine them to provide a consistent comparison between treated and untreated Bambara groundnuts. H_2O-LPP treatment introduced hydroxyl functionalities that improved the wettability of the Bambara groundnut seeds [25]. It is also likely that the carbon moieties associated with C-N increased during H_2O-LPP treatment, as suggested by the component fitting of N1s spectra. Finally, the nitrogen on the Bambara groundnut also oxidized from amine to amide during the H_2O-LPP treatment (Figure 9).

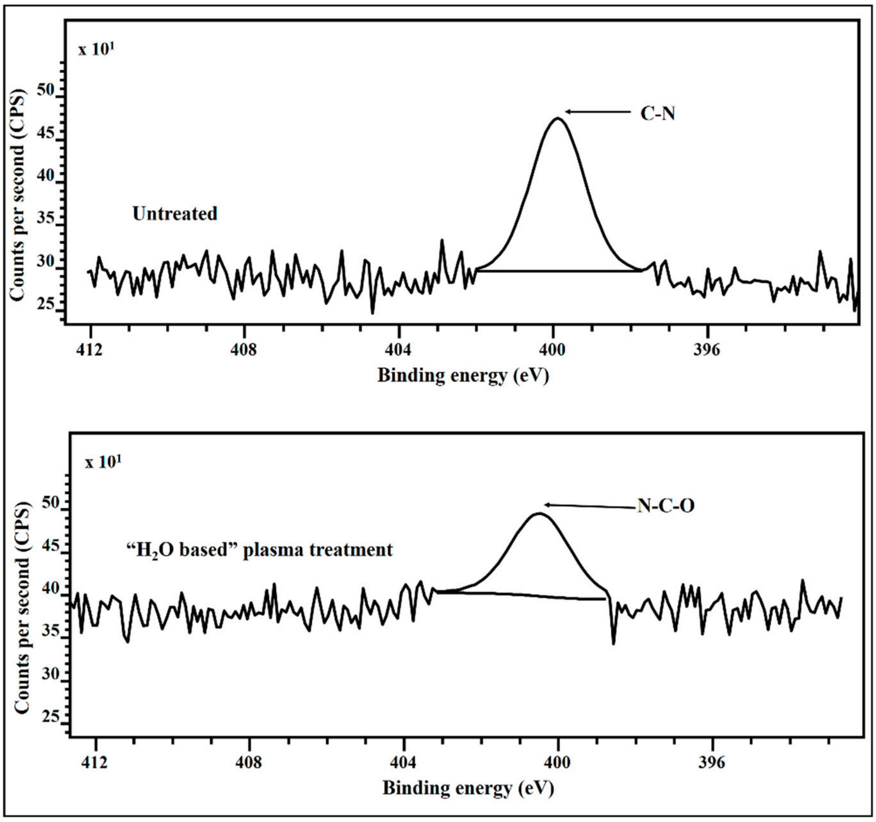

Figure 9. Component fitting of high-resolution N1s spectra of untreated and 10 s H_2O-LPP-treated Bambara groundnut seeds.

3.8. Optical Emission Spectroscopy (OES)

Optical emission spectra were monitored in the spectral range of 350–950 nm, as shown in Figure 10. This monitoring revealed the dominance of oxygen (673 and 777 nm), hydrogen (656.5 and 866 nm), nitrogen (750 and 375 nm), and hydroxyl (727 nm) species [49]. Nitrogen species originated from the relatively high vacuum level of the plasma reactor; the hydroxyl groups were likely to be responsible for the etching on the seed coats. Our results coincide with others that confirmed similar active ion species as interacting with artichoke seeds, leading to fast germination and growth [50]. Our OES results also dovetail with our XPS analysis of LPP-treated seeds, which exhibited similar chemical functionalities. These chemical changes on the seeds facilitated water uptake and altered the WCA, which stimulated seed germination.

In short, plasma treatment enriched the surface of Bambara groundnut seeds with oxygen-containing functional groups; this enrichment improved the seed wettability, which accelerated germination [15,35,51]. Furthermore, the newly formed oxygen functional groups also played an essential role in the stimulation of germination-related biochemical processes [51].

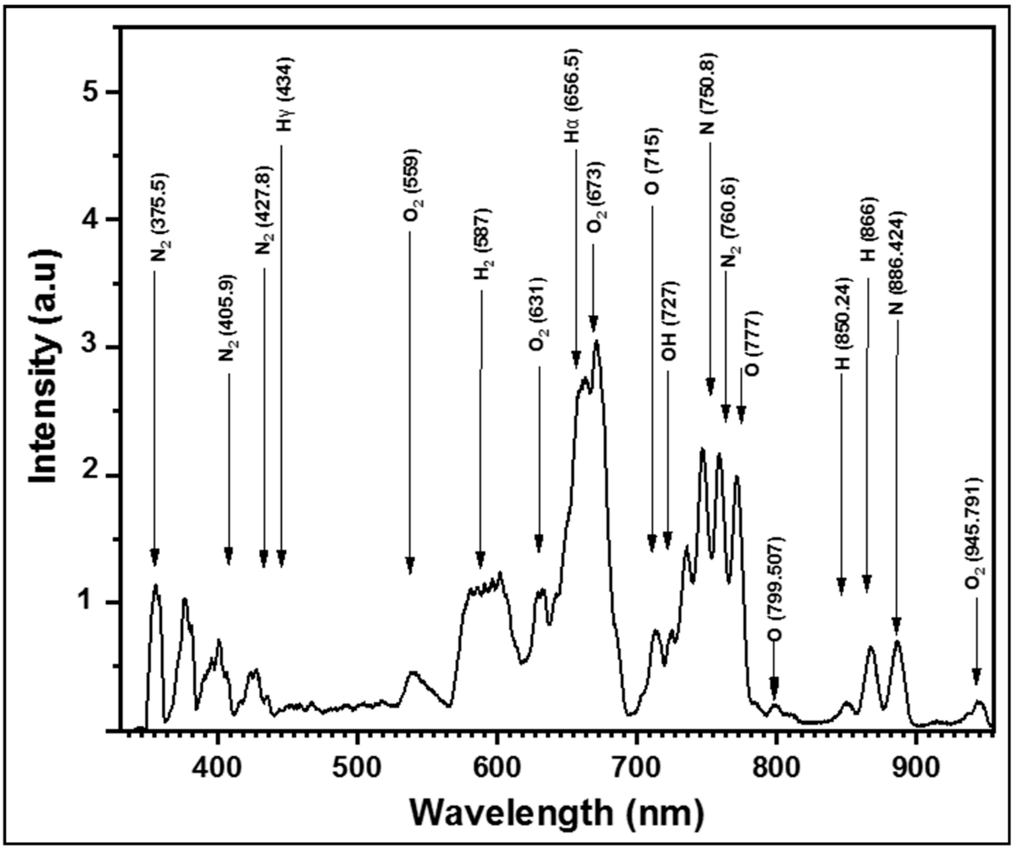

Figure 10. Optical spectrum emitted from low-pressure water plasma at a power of 10 W.

4. Conclusions

H_2O-LPP treatment improved the Bambara groundnut seed germination significantly compared to untreated seeds, as illustrated by various germination-related parameters (i.e., germination potential, germination %, root length, shoot length, and seed vigor index). The most significant improvement in seed germination resulted from the treatment parameters of 10 W 10 s, while more aggressive treatment (30 W 10 s) did not yield any significant differences from untreated seeds because the high plasma dose cracked and damaged the seeds. During plasma treatment, plasma ions (primarily hydroxyl ions) etched the seed hilum and made it porous to water imbibition, as measured by the water uptake, thereby encouraging the activation of growth hormone and germination. The plasma etching also released other ions and solutes, as detected by electrical conductivity tests, and improved the wettability of the seeds, as shown by the reduction in the apparent water contact angle, thereby accelerating water imbibition. FESEM imaging confirmed the uneven etching of the seed hilum, and the reduction in apparent WCA was likely attributable to the oxidization of the seed surface, which produced hydrophilic ions that improved the seed wettability.

While the H_2O-LPP treatment proved effective in improving the Bambara groundnut seed germination, the associated biological mechanisms at the cellular level remain unclear beyond the physical etching and chemical functionalization described here. The lack of hydrophobic recovery observed in the WCA studies proved useful in ensuring the effects of treatment persisted until the seeds were eventually planted; however, further studies elucidating the mechanics behind these twin features of biological changes and the lack of hydrophobic recovery are essential to boost the confidence in scaling up this LPP technique to broader use. In addition, we are also looking into transferring this new germination understanding from low-pressure to atmospheric-pressure plasma to improve the scalability of this plasma treatment.

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