Effects of Atmospheric-Pressure N\textsubscript{2}, He, Air, and O\textsubscript{2} Microplasmas on Mung Bean Seed Germination and Seedling Growth

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Atmospheric-pressure N\textsubscript{2}, He, air, and O\textsubscript{2} microplasma arrays have been used to investigate the effects of plasma treatment on seed germination and seedling growth of mung bean in aqueous solution. Seed germination and growth of mung bean were found to strongly depend on the feed gases used to generate plasma and plasma treatment time. Compared to the treatment with atmospheric-pressure O\textsubscript{2}, N\textsubscript{2} and He microplasma arrays, treatment with air microplasma arrays was shown to be more efficient in improving both the seed germination rate and seedling growth, the effect attributed to solution acidification and interactions with plasma-generated reactive oxygen and nitrogen species. Acidic environment caused by air discharge in water may promote leathering of seed chaps, thus enhancing the germination rate of mung bean, and stimulating the growth of hypocotyl and radicle. The interactions between plasma-generated reactive species, such as hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and nitrogen compounds, and seeds led to a significant acceleration of seed germination and an increase in seedling length of mung bean. Electrolyte leakage rate of mung bean seeds soaked in solution activated using air microplasma was the lowest, while the catalase activity of thus-treated mung bean seeds was the highest compared to other types of microplasma.

Non-equilibrium low temperature plasmas have been attracting significant attention in material fabrication\textsuperscript{1–4} and more recently in medicine and biotechnology for their ability to induce desirable biochemical responses in living organisms, with potential applications ranging from selective cancer treatment\textsuperscript{5,6}, wound healing\textsuperscript{7}, surface and solution disinfection and decontamination\textsuperscript{8}, to sustainable agriculture\textsuperscript{9–13}. In the case of the latter, the non-ionizing low-level radiation and numerous reactive species, including reactive oxygen and nitrogen species (ROS and RNS) generated by plasma can be used to induce desirable changes in a broad spectrum of developmental and physiological processes in plants, improving seed resistance to stress and diseases, modifying seed coat structures, increasing the permeability of seed coats, and stimulating seed germination and seedling growth\textsuperscript{14–16}. These desirable effects were demonstrated in several types of commercially significant food plants for human and animal consumption, such as wheat\textsuperscript{11,17}, barley\textsuperscript{18}, tomato\textsuperscript{12,19}, soybean\textsuperscript{10,20} and thale cress (Arabidopsis thaliana)\textsuperscript{21}. For example, recent studies by Koga \textit{et al.} showed that a single 3-minute treatment of dry seeds of Arabidopsis thaliana led to growth acceleration in all the growth stages, including shorter harvest period, a considerable increase in total seed weight, an increase in each seed weight, and a substantial increase in seed number\textsuperscript{21}. Although the specific mechanisms by which plasma-generated physical and chemical effects influence the metabolic activity of the seed or the plant remain poorly understood, changes in morphological and sowing features of seeds\textsuperscript{11}, dehydrogenase activity, superoxide dismutase (SOD) and peroxidase activity, photosynthetic pigments, photosynthetic efficiency and nitrate reductase activity have been reported\textsuperscript{12}.

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One of the key reported advantages of plasma seed and plant treatment is that favorable biological responses can be induced in the absence of potentially environmentally-harmful chemicals, which makes plasma-based treatment a more environmentally-sustainable alternative to traditional chemical pathways used to improve seed performance and crop yield. Li and Jiang et al. reported that an 80 W cold RF plasma treatment significantly improved seedling growth, including shoot length, shoot dry weight, root length and root dry weight10, and Edward et al. reported that the yields of lentils, bean and wheat significantly improved as a result of the cold RF plasma treatment induced oxidation of seed surface and generated nitrogen containing groups11. Zhou and Huang et al. showed that the effect of plasma treatment on the specific traits and yield of tomato was voltage-dependent and plasma treatment at 6120 V produced best results22. However, to date, the majority of the reported plasma treatment centered on the use of low-pressure radio frequency systems10,23,24, which have some obvious limitations in terms of real-life use, specifically with regard to the environmental and economic costs and processing restrictions associated with vacuum processing. This limitation can be addressed by atmospheric-pressure plasmas that are able to produce a wide range of reactive chemical species and physical effects under vacuum-free conditions. Currently, there is a growing number of research groups in USA25, Germany26, Japan27, Australia28 and others, that investigate the use of low-temperature (“cold”) atmospheric-pressure plasmas (CAP) on seed metabolism.

Mung bean (Vigna radiata (Linn.) Wilczek.) is an important economic crop in South East Asia. Diseases and abiotic stresses, such as drought, heat, water logging and salinity, can lead to a considerable loss in nutritional quality and economic yield of mung bean29. These issues are traditionally addressed through genetic engineering and use of growth-inducing chemicals. However, there are two critical issues associated with the use of conventional antibiotics to treat agriculturally-relevant pathogens. The first one is that, just as the case with human pathogens, excessive antibiotic treatment can induce the development of antibiotic resistance, decreasing the effectiveness of not only this therapy but other therapies that share the same microbial target. The resistant pathogen can also transfer the relevant genes to other pathogenic microorganisms, including those that present danger to animals and humans. The second concern involves the unintentional transfer of the sub-inhibitory quantities of antibiotic to the environment, including other plants, animals and humans. In both cases, replacement of the antibiotic with an alternative therapy is beneficial.

Cold atmospheric plasma (CAP) is a new, promising antibacterial treatment to combat antibiotic-resistant bacteria with synergies that arise from chemical species and physical effects. Matthes et al. reported that repeated applications of cold atmospheric pressure plasma on Staphylococcus aureus embedded in biofilms did not result in the development of resistance or habituation against plasma applied within short time periods30. On the other hand, Mai-Prochnow et al. reported that a short plasma treatment (3 min) of Pseudomonas aeruginosa embedded into biofilms may lead to the emergence of a small number of surviving cells exhibiting enhanced resistance to subsequent plasma exposure31.

This study aims to investigate CAP treatment as potential means for enhancement of productivity, specifically seed germination and seedling growth of mung bean crop. Using a custom-built system (Fig. 1), the mechanisms of CAP interactions with the mung bean at different stages of bean development will be studied.

Results

Seed germination percentage. Figure 2 shows the typical seed morphology and the germination percentage of mung bean seeds treated with different types of plasma as a function of incubation time. The seed germination percentage was strongly dependent on the incubation time and the feed gas used. As the data from repeated experiments suggest, the germination percentage increased with incubation time, which was translated into a line graph (see Fig. 2(e)). Among tested microplasma arrays, the air and O2 microplasma arrays were more efficient in enhancing the seed germination, which could be attributed to the relatively high density of reactive oxygen species generated inside air and O2 microplasmas32. After incubation for 12h, seed germination rate of
mung bean treated with the air plasma reached 80%, significantly higher than that reached by seeds treated with O₂ microplasma (15%) and He and N₂ plasmas (below 10%). After incubation for 24 h, the germination percentage of air plasma treated samples reached approximately 95%, whereas the corresponding value for O₂ plasma treated seeds ascended to 72%. Seeds treated by He and N₂ plasma had the lowest germination rate of 30%, almost the same as that for control samples. Finally, after incubation for 48 h, almost all of the treated and control mung bean had germinated.

Seed germination (germination potential and germination index) and seedling growth (plant length and length index). Germination potential, germination index are the most significant parameters of biological vigor of the seed. Figure 3 shows that germination potential, germination index, plant length and length index of mung bean seeds were influenced by CAP differently depending on the nature of the gas used to generate the plasma. There were no significant differences in germination potential between samples treated with
N2, He, Air, O2 microplasmas and those in the control group (see Fig. 3(a)), since almost all the seeds germinated after 2 days regardless of being treated or not. Figure 3(b) shows that air plasma treatment significantly increases germination index of mung bean seeds from 60 to 95. The germination index of mung bean seeds treated with O2 plasma was slightly lower, at 85. Compared with the control, the air and O2 plasma treatments significantly increased the germination index by 58.3% and 41.7%, respectively. On the other hand, there was no significant difference between germination indices of seeds treated with He or N2 plasma and that of the control. Overall, air plasma treatment produced the most favorable combination of germination potential, germination rate and germination index of mung bean seeds, suggesting that the cocktail of reactive species produced by this type of plasma under these experimental conditions is best suited to promoting seed germination outcomes of mung bean.

Figure 3(c) shows the effect of CAP treatment on the length of mung bean sprouts as a function of gas used for plasma treatment. Sprouts grown from air plasma-treated seeds had achieved the longest plant length within the incubation time (24–96 h). The plant growth of O2 plasma-treated samples was slower, while the N2 and He plasma-treated samples displayed sprout lengths similar to those grown from control seeds. After 24 h of incubation time, the plant length of air plasma treated samples reached approximately 10 mm, 3–5 mm longer than that of mung bean treated with other types of plasmas or the control. With the increase of incubation time to 96 h, plants within the air and O2 plasma-treated groups reached 67.5 mm and 47.4 mm in length, respectively, while He plasma and N2 plasma-treated samples displayed only marginally higher plant lengths than those in control group. Figure 3(d) shows that the respective length indices of mung beans treated by the O2, N2 and He plasma were 72.3%, 25.1% and 24.9% higher compared to the control, while the corresponding value for air plasma treatment was estimated to be approximately three times that of the control. These results indicate that seeds treated by air discharge not only had better germination performance, but also had a higher growth activity and length index.

**pH value of the plasma treated solution.** The pH values of the seed-containing solutions after 10 min of plasma treatment with N2, He, air, and O2 as feed gas, were measured, as shown in Table 1. All the plasma treatments were performed at $V_p = 4.5$ kV. Treatments with atmospheric-pressure air and N2 plasma arrays resulted in a slight decrease in the pH value of the solution. This was attributed to the effects of nitric and nitrate acids produced from the reaction of H2O molecules with NOX species, which were generated in the air microplasmas. The pH values of the solutions treated by O2 and He microplasmas increased only slightly. One possible explanation is that energetic collisions of electrons with water vapor molecules can result in the formation of OH species in water and thus lead to an increase in the pH value. Previous studies also showed that mung bean seeds treated by slightly acidic electrolyzed functional water presented faster growth than those treated with tap water due to the low electrolyte leakage rate and high catalase activity observed in the former.

**Concentration of plasma-generated H2O2 molecules and nitrogen-containing species.** The potential of N2, He, Air and O2 plasma treatments to induce changes in the concentration of H2O2 radicals in distilled water was investigated as a function of the treatment time (Fig. 4(a)). All atmospheric-pressure microplasmas used in this experiment were generated at a $V_p$ of 4.5 kV, corresponding to a discharge power of 25 W. Overall, the H2O2 concentration increased with the duration of plasma treatment time. This increase was attributed to the high electron density, energy of the plasma and long life time of the excited species that facilitate the energy transfer between the excited plasma species and water molecules, leading to H2O2 formation (*e− + H2O → H2O2 + H+ + e−*).

| Discharge gas | Air | O2 | N2 | He |
|---------------|-----|----|----|----|
| pH value of solution | 5.1 | 7.2 | 6.8 | 7.5 |

Table 1. The pH values of the solutions after 10 min of plasma treatment with N2, He, air, and O2 as feed gas.

![Figure 4](https://example.com/fig4.png) Concentrations of (a) H2O2 and (b) NO2− and NO3− in aqueous solution treated with N2, He, Air, or O2 microplasmas as a function of the treatment time.
Among these four types of plasmas, the air microplasma treatment showed the highest H₂O₂ concentration (17.4 mg/liter) in the 10 min plasma-treated solution, while the H₂O₂ concentrations in the O₂, He and N₂ microplasma-treated solution were relatively low (7.9 mg/liter, 1.2 mg/liter and 0.5 mg/liter, respectively). The significantly higher H₂O₂ concentration in the sample treated with air microplasma may be due to air discharge being more conducive to the formation of OH radicals. On the other hand, as an electron-negative gas, O₂ discharge results in the formation of an excess of oxygen containing species that can adsorb electrons by direct electron attachment (O₂ + e− → O₂−) or dissociated attachment (O₂ + e− → O + O²−), consuming the electrons that would otherwise participate in H₂O₂ formation. In He and N₂ discharges, H₂O₂ molecules are produced solely via the collision between energetic electrons and H₂O molecules, resulting in a significantly lower concentration of H₂O₂ in solutions treated with these microplasmas.

The formation of nitrite (NO₂⁻) and nitrate (NO₃⁻) in the plasma-treated solution is illustrated in Fig. 4(b), which shows the production of some long-lived and relatively stable chemical species in water as a result of air plasma treatment. NO₂⁻ and NO₃⁻ are formed in plasma-treated water through the dissolution of nitrogen oxides formed in the plasma by gas-phase reactions of dissociated N₂ and O₂ or H₂O. Results show that NO₂⁻ and NO₃⁻ were formed in water with the constant rate following zero-order rate kinetics indicating a direct effect of the plasma. Among these four types of plasmas, the air microplasma treatment showed the highest NO₂⁻ and NO₃⁻ concentration (1.2 mg/liter) in the 10 min plasma-treated solution, followed by that for N₂ microplasma, O₂ microplasma, He microplasma listed in decreasing order. Moreover, along with the formation of NO₂⁻ and NO₃⁻ in the plasma-treated water, the dissolution of NO₂ in water produces H⁺ ions following the reaction NO₂(aq) + NO₃(aq) + H₂O(l) → NO₂⁻ + NO₃⁻ + 2H⁺, which is consistent with the measured pH values.

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**Surface physico-chemical properties of mung bean seeds.** Scanning electron microscopy (SEM) images of seed coat surface were used to examine the effect of plasma treatment on the morphological characteristics of mung bean seeds. As shown in Fig. 5, surface structure of seeds changed sharply as a result of air plasma treatment. Figure 5(a) indicates that the surface topography of control bean seeds was comprised of irregular rhopyshaped features with the size varying from 1.0 to 3.0μm. The surface structure of N₂, He, and O₂ plasma treated seeds did not undergo dramatic changes, and displayed similar topography to that of control samples. By contrast, the air plasma-treated seeds had an eroded surface, with no significant ridges (Fig. 5(b)). These results indicate that acidic environment caused by air discharge in water may have contributed to the chapping of seed coat. After the air plasma processing, the highly compact surface texture of the seed coat may be more fragile and hence easier to crack in acidic plasma-activated water, which would facilitate the more efficient absorption of water and nutrients, and consequently enhance the germination rate and promote the growth of hypocotyl and radicle of the treated mung bean seed. The wettability of seeds can be reflected by the apparent contact angle which results from a complex interplay between chemical composition and roughness of the surfaces. The apparent water contact angles on surfaces of the He, N₂, and O₂ plasma-treated seeds were similar to that of the control, at 56.4° (Fig. 5), since these plasma treatments did not significantly alter the topography of the seeds. The smallest apparent contact angle was obtained on surfaces of seeds treated with air plasma (Fig. 5(g)), attributed to the plasma-induced changes to the chemical structure and the surface topography of the seed surface. The resulting increased wettability of the air plasma-treated seeds may be partially responsible for the observed increase in the uptake of water.

**Effects of H₂O₂ concentration on seed germination and seedling growth.** As shown in Fig. 6(a), H₂O₂ played a positive role in accelerating the germination of mung bean. Compared to the control, the six H₂O₂ solutions with concentrations ranging from 0.01% to 0.30% all contributed to higher germination rates within fixed incubation time. However, there was an inverse relationship between H₂O₂ concentration and the germination rate. During the first 12 h of incubation, mung bean treated with 0.01%, 0.03% and 0.05% of H₂O₂ displayed dramatic increases in germination rate, from fairly low levels to more than 60%, while no germination was observed in the control. After 48 h of incubation, both control samples and H₂O₂-treated mung bean germinated entirely. The curves presented in Fig. 6(c) show the relationship between H₂O₂ concentration and plant length. When the concentration of H₂O₂ was below 0.07%, the treatment was highly conductive to plant growth, and the 0.01% H₂O₂ treatment outperformed the others at any incubation time. However, when the concentration of H₂O₂ was drastically increased to 0.10% and 0.30%, the growth of mung bean was hindered. As mentioned above, 0.01% H₂O₂ solution acted as a significant motivator for both germination rate and plant length of mung bean. To further explore the phenomenon, Fig. 6(b,d) were presented to make comparisons between 0.01% H₂O₂ treated and air plasma-treated samples with respect to their ability to improve the germination rate and plant length, respectively. While the results of the two treatments were similar, air plasma treatment was slightly more effective, especially in boosting plant growth. The disparity implied that although H₂O₂ was a major factor in promoting mung bean germination and growth, other plasma-generated factors may have contributed, with potential yet to be fully explored synergies that may arise from distinct plasma effects. As previously mentioned, in addition to a rich mixture of chemical species, plasma generates photons, electric fields, shock waves, etc. For example, formation of solvated electrons at a plasma-solution interface opens questions about their behaviour in the presence of strong electric fields, as suggested by the blue-shifted absorption spectrum.

**Effects of LNF solutions and air plasma treatment time on seed germination and seedling growth.** One well-known fact is that nitrogen-containing species such as NO₃⁻ and NO₂⁻ are generated in air plasma. In view of this, it might prove instructive to analyze the effects of nitrogen on plant growth as nitrogen is one of the essential nutrient elements in the plant growth. In this experiment, an aqueous solution containing...
0.1–3.0 g/L of NaNO₃ and NaNO₂ was used to represent liquid nitrogen fertilizer (LNF) to study the effects of nitrogen on the germination and growth of mung bean, and the results were shown in Fig. 7. Clearly, LNF (0.1 g/L to 3.0 g/L) increased the germination rate of mung bean, with the most significant improvement observed in seed groups treated with solutions containing 0.3 g/L LNF. The influence of LNF on plant length (presented in Fig. 7(b)) was similar to that of H₂O₂ (shown in Fig. 6(c)), with solution containing lower concentrations of the fertilized (0.3 g/L LNF) creating the most favorable conditions for mung bean growth. Another point that should be noted is that highly concentrated LNF would be detrimental or even fatal to plant growth. This leads us to the conclusion that nitrogen may play a critical role in the air plasma-stimulated germination and growth of mung bean. The effect of air plasma treatment time on germination rate and plant length was also investigated. Figure 7(c,d) shows the effect of air plasma treatment duration on the germination rates and plant growth of mung bean seeds measured as a function of incubation time. Clearly, both the germination percentage and plant growth were strongly dependent on the air plasma treatment time. Although moderately extending the treatment time led to a significant increase in the germination rate and seedling growth of mung bean, this upward trend was restrained when the air plasma treatment was over 15 min. This is mainly because prolonged plasma treatment might result in an increase in the temperature of the solution, adversely affecting plant growth.
Discussion

Reports have shown that some reactive species generated in plasma gas phase cannot penetrate the gas-liquid interface (several μm to hundreds of μm) or diffuse into the solution within their short life time during the plasma treatment. In general, only a small portion of species, such as O₃, H₂O₂, H, OH, NOₓ and HNOₓ, can pass through the gas-liquid interface and enter the solution. Compared with other radicals, H₂O₂, NOₓ and HNOₓ exist in the solution for a longer period of time.

These reactive oxygen species (ROS) and reactive nitrogen species (RNS), as evidenced by recent studies, play an important role in cell proliferation, differentiation and apoptosis and can function as signaling molecules. It was detected in our experiment that air plasma generated RNS radicals (nitrogen oxide (NOₓ) molecules, HNOₓ) was in part responsible for the observed acidification of the solution (pH < 7). Acidification of plasma-activated water contributed to the chapping of the waxy layer in the seed coat (see Fig. 5), which in turn promoted the ability of the treated seeds to absorb water and nutrients, increased the germination of mung bean, and accelerated the growth of hypocotyl and radicle. In addition, mung bean seeds treated by air plasma in water had a lower rate of electrolyte leakage, making it possible for the seeds to maintain a relatively high activity.

Nitrogen, in particular, is indispensable for plant growth. Under natural conditions, nitrogen bound in soil mainly exists in four types of compounds - ammonium salts (NH₄⁺), nitrates (NO₃⁻), proteins and products of protein decomposition (amino acids, amines, peptides and humus compounds). It is justifiable to assume that a favorable environment for mung bean seed germination might occur in solutions containing a proper source of nitrogen, thus improving the nutritional values of the solutions.

Using LNF as feed can increase nitrogen accumulation in the mung bean plant and improve the activity of nitrate reductase and glutamine synthetase related to nitrogen metabolism and photosynthesis, which contributes to the growth of seedling. However, excessive use of LNF will give rise to the disorder of nitrogen metabolism in bean plants, inhibiting mung bean nodule formation and symbiotic nitrogen fixation. Another reason for the reduced rate of canopy photosynthesis under high nitrogen may be that the overdose of nitrogen produces toxic organic nitride, the presence of which damages plant growth.

The effects of different gas discharge treatment on the electrolyte leakage rate of mung bean were investigated and the results are shown in Fig. 8(a). Among all treated seeds, air plasma-treated samples had the lowest electrolyte leakage rate and therefore the highest metabolic activity, so unsurprisingly their hypocotyls were the longest. Compared with air plasma-treated mung bean seeds, those subjected to O₂ plasma treatment presented
a slightly higher electrolyte leakage rate, while the other two treatments showed little difference to the control in this respect. Figure 8(b) shows the effects of different gas discharge treatment on the catalase activity of mung bean. Catalase can remove H$_2$O$_2$, and is part of the defense system, so the catalase activity is highly interrelated with the ability of plants to tolerate stress. Low catalase activity would lead to the accumulation of H$_2$O$_2$ in plant cells as well as disruption of metabolic activity. It is clearly seen from the chart that catalase activity of the air plasma treated seeds was 21.9% higher than the corresponding value for the control, which means that air plasma treatment can benefit plant growth by increasing its ability to resist/tolerate stress.

Generally, not all active oxygen species are detrimental, and not all antioxidants are beneficial. Balancing the production and clearance of reactive oxygen species is vital to the plant’s growth and metabolism and its ability to
respond to environmental stresses. After a long history of evolution, plants have formed effective mechanisms of active oxygen scavenging which can be divided into two categories: enzymatic and non-enzymatic. The first group includes such enzymes as superoxide dismutase, peroxidase, glutathione peroxidase and ascorbate peroxidase, whereas the non-enzymatic group includes ascorbic acid, carotenoids and flavonoids. In addition to clearing up the ROS through chemical reactions, these substances can also act as a substrate for the enzyme, boosting the active oxygen scavenging. Since H₂O₂ treatment enhances the activity of peroxidase, ascorbate peroxidase and ascorbate oxidase, while reducing ascorbic acid and zeatin, plasma treatment that delivers sufficient quantities of exogenous H₂O₂ to mung bean seeds may effectively increase the oxygen scavenging ability of the plant and thus increase seed germination rate and promote the growth of mung bean seedlings, as shown in Fig. 6.

Plasma treatment can indeed provide a chemical-free means of stimulating seed germination and plant growth. However, to achieve considerable improvement in agricultural efficiency, the enhancement should be preferably maintained throughout the growth cycle in its entirety, leading to higher productivity, i.e. faster harvest, higher weight per fruit or seed, and more numerous fruit or seeds, as well as higher quality, more nutritious and tasty fruit or seed. Recent evidence suggest that valuable plasma effects are indeed retained throughout the growth cycle and even potentially passed on to future generations via pathways other than genetic mutations.

**Conclusion**

In this study, investigations of the seed germination and seedling growth rates of mung bean were performed by using atmospheric-pressure N₂, He, air and O₂ microplasma arrays in water. Compared to the O₂, N₂ and He microplasma treatment, the air microplasma treatment was more effective in enhancing seed germination and seedling growth of mung bean in aqueous solution. Some exogenous experiments including treatment by H₂O₂ solution and LNF solution were performed to study the mechanisms of plasma-generated species interactions with the mung bean. Analysis showed that the ROS and RNS species generated by air plasma in solution played a critical role in the germination and growing process. Our research shows the feasibility and advantages of cold plasma application to seed treatment, and also provides theoretical basis for the utilization and popularization of this technique.

**Methods**

Atmospheric-pressure microplasma array is used to treat mung bean seeds in aqueous media, as shown in Fig. 1(a). The seed treatments were carried out at the Institute of Physics and Mechanical & Electrical Engineering, Xiamen University, Xiamen, China (118°06′E, 24°27′N), from March to September, 2015. 100 uniform seeds of mung bean (obtained in Nanjing City, Jiangsu, China) were overspread on a filter screen which was placed 1 cm above the microplasma jet units in the plasma processing system. The seeds were then exposed to inductive air plasma generated in solution with Dielectric Barrier Discharge (DBD) for 10 min. Meanwhile, the same number of seeds in the control group were also subjected to the same plasma reactor and feed gas flux for 10 min in the absence of plasma. After 10 min of plasma treatment, the treated seeds were placed on the filter cloth in 9 cm petri dishes and 10 mL of distilled water was added into each dish to create germinating conditions. After that, these samples were incubated in a light incubator at the temperature of 25 °C. During the germination and growth, 5 mL of distilled water was added daily to each petri dish to keep sufficient moisture for germination. The germination percentage was recorded every 3 hours for 4 days. The morphological measurements of mung bean sprouts were performed at the 12 h intervals after germination began. The total length of mung bean sprouts, including the length of hypocotyls and the length of radicles, was measured by a ruler, as shown in Fig. 1(b). Every reported measurement represents the average length of 25 sprouts per treatment group.

The concentration of hydrogen peroxide in the plasma-treated water was determined by color forming reactions and spectrophotometric measurements. When titanium oxysulfate (TiO(SO₄)₂) reacts with H₂O₂, a yellow-colored complex (pertitanic acid) was formed and UV–Vis measurement was done at 407 nm to colorimetrically determine the concentration of H₂O₂ (TiO²⁺ + H₂O₂ → [Ti(OH₂)₅]²⁻)³⁸,⁵⁸. For nitrite and nitrate detection, the well-known Griess assay was used to estimate the concentrations of nitrates (nitrates are first reduced to nitrites), which can react with Griess Reagents to form a deep purple azo compound whose absorbance at 550 nm can be measured. Electolyte leakage rate and catalase activity in seeds were recorded immediately after air plasma treatment. It should be noted that all the seed experiments reported in this letter were planned as a completely randomized design with three replications, and the results are consistent under the same experimental conditions. Several parameters were used to describe the statistical characteristics of seeds:

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\text{Germination percentage} = \frac{\text{total number of germinated seeds}}{\text{total number of seeds}} \times 100
\]  
\[
\text{Germination potential} = \frac{\text{number of seeds germinated in 3 days}}{\text{total number of seeds}} \times 100
\]
Germination index\(G_i = \sum \frac{N_i \times \text{(number of germinated seeds on the t day)}}{D_i \times \text{(germination days)}}\) (3)

Length index = Germination index \times \text{total length.} (4)

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Author Contributions
Renwu Zhou, Rusen Zhou, X.Z. and J.Z. initiated the research, worked on plasma treatment, and performed the experiments. S.Y., K.O. and K.B. advised on planning and executing the research. All authors discussed the results. R.Z. and K.B. wrote the manuscript.

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