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Atmospheric-Pressure Plasma Treated Water for Seed Germination and Seedling Growth of Mung Bean and Its Sterilization Effect on Mung Bean Sprouts

Jiangwei Li[a,+], Renwu Zhou[a,b,+], Rusen Zhou[b,*], Xianhui Zhang[a,c*], Size Yang[a]

[a] Department of Electronic Science, College of Physical Science and Technology, Fujian Provincial Key Laboratory of Plasma and Magnetic Resonance, Institute of Electromagnetics and Acoustics, Xiamen University, Xiamen 361005, China

[b] School of Chemistry, Physics and Mechanical Engineering, Queensland University of Technology, Brisbane, Queensland 4000, Australia

[c] Shenzhen Research Institute of Xiamen University, Xiamen 361005, China

[+] These authors contributed equally to the work.
[*] Corresponding author, zhourusen2008@gmail.com (R.S. Zhou) and zhangxh@xmu.edu.cn (X.H. Zhang).

ABSTRACT

Plasma treated water (PTW), produced by atmospheric-pressure plasma treatment of water, usually contains various reactive oxygen and nitrogen species (RONS). This study aimed at evaluating the effectiveness of different types of PTW on seed germination, seedling growth and microbial sterilization during the germinated mung bean processing. Results showed that air-PTW possessed outstanding abilities in improving seed germination and seedling growth with a germination index of 95.50% and a vigor index of 1146.64, and in microbial decontamination. The physicochemical properties of the PTW were analyzed to better understand the PTW stressed germination. Some physiological parameters like the activity of superoxide dismutase (SOD), the contents of malondialdehyde (MDA)) and phytohormone (indole acetic acid (IAA) and abscisic acid (ABA)) during germination were also evaluated. This study suggested that air-PTW treatment could indeed provide a green and effective mean of stimulating seed germination and plant growth, and thus accelerate the growth cycle.

Industrial Relevance: Increasing the production of food by using both economical and environmentally friendly means has been deemed as an urgent matter to sustain the food demand of rapidly growing world population. The results of this study suggest that PTW presents a great opportunity to address this need by increasing seedling growth and viability. PTW treatment is an environment-friendly and low-cost mean of stimulating seed germination and plant growth, which possesses the potential of scale up or industrial applications in relevant fields.

Keywords: plasma-treated water, mung bean, seed germination, seedling growth, sterilization

1. Introduction

Mung bean (*Vigna radiate* (L.) R. Wilczek), an important leguminous crop, is widely cultivated and used in kinds of cuisines across Asia and some parts of South and North America and Australia, and is rich in nutrients such as protein and iron (Peñas, Gómez, Frías, & Vidal-Valverde., 2010; Dahiya et al., 2015). After soaking mung bean in water and incubating under certain temperature and humidity conditions for several hours, the germinated mung bean named mung bean sprouts can be harvested. Germinated mung bean sprouts are popular oriental vegetables vended in public markets because of wide availability aAaand high nutritional and medicinal values (Gabriel et al., 2007; Mubarak, 2005). The sprouts are available all year round and good sources of dietary proteins, carbohydrates, minerals and vitamins comparable to those found in the more expensive animal and marine sources (FNRI, 1997).

Nevertheless, many factors can result in a considerable loss in production, nutritional value and economic yield of mung bean sprouts, especially microbial diseases and physiological deterioration (Randeniya & de Groot, 2015). Various methods such as genetic engineering, physical intervention technologies and chemical addition have been developed to achieve considerable improvement in agricultural efficiency. Ashraf & Akram (2009) suggested that genetic engineering, an alternative strategy to conventional breeding, was being employed emphatically worldwide these days not only for improving stress tolerance but also for improving the quality and yield potential of most crops. Nei & colleagues (2010) proved that the combination of low-dose irradiation with acidified sodium chlorite showed good sterilization effectiveness during the germination of mung bean. Kasprowicz-Potocka et al. (2013) reported that additional sources of nitrogen, carbon and sulphur can be used in the germination process for protein synthesis, and in particular for the synthesis of sulphur amino acids, which considerably determines the nutritional value and, as a result, the feed usefulness of lupin sprouts. However, the downsides of these methods should not be ignored, including the high fixed costs and complexities of production process, the developed agriculturally-relevant pathogens with antibiotic resistance which can also transfer the relevant genes to other pathogenic microorganisms, and the induced danger to other plants, animals and humans (Gabriel et al., 2007). Therefore, improvement of the existing techniques with ecologically safe, economical and effective ways to improve seed performance and crop yield is required.

Atmospheric-pressure plasma (APP), which is defined as a partially ionized gas, consisting of charged particles, reactive species, electric fields, and ultraviolet photons, has been widely considered to be an effective method for inactivation of microorganisms (Lu, Liu, Song, Zhou, & Liu, 2014; Zhang et al., 2014; Zhou et al., 2015). Reactive species, reactive oxygen and nitrogen species (RONS) in particular, are generally considered to be responsible for the antimicrobial efficacy (Zhang et al., 2016; Zhou et al., 2015). Recently, it has been confirmed that the reactive species generated by APP could react with water to produce PTW possessing outstanding antibacterial ability, which could efficiently inhibit a wide range of microorganisms, including *Escherichia coli, Saccharomyces cerevisiae, Hafnia alvei, Staphylococcus aureus,* and *Candida albicans* (Kamgang-Youbi et al., 2008; Kamgang-Youbi et al., 2009; Naïtali et al., 2010; Oehmigen et al., 2010; Traylor et al., 2011). Due to superior advantages such as less adverse impact on the environment and no need for transportation and storage of potentially hazardous chemicals, especially chlorine-based products, PTW is deemed to be a green disinfection product and a promising alternative to traditional sanitizers applied in biomedical and environmental fields. However, to the best of our knowledge, there are few reports on the application of PTW to improve both the seed germination rate and seedling growth of mung bean, and exploration of the relationship between antioxidant enzyme activity, phytohormones contents and mung bean sprouts growth during PTW-induced germination.

In this study, one home-made plasma jet was designed and used to produce PTW with four commonly processing gases. The present work aimed at exploring the effects of N_2-PTW, He-PTW, Air-PTW, and O_2-PTW on seed germination and seedling growth of mung bean, and their effects on mung bean sprouts sterilization. The ORP, electrical conductivity, pH and the different concentrations of plasma-generated long-lived chemical species (NO_3-, NO_2- and H_2O_2) in PTW were recorded to estimate the physicochemical properties of these types of PTW. With respect to the mung bean crop, parameters of seed germination percentage, germination potential, germination index, plant length and the diameter of hypocotyls were evaluated. Colony-forming unit (CFU) was used to assess the sterilization efficiency of PTW on mung bean sprouts. Moreover, in order to deepen knowledge of the physiological processes in mung bean sprouts when treated by different types of PTW, the activity of antioxidant enzyme (superoxide dismutase (SOD), an important antioxidant enzyme in almost organisms, and malondialdehyde (MDA), a common index in plant senescence physiology and resistance physiology research), and typical phytohormones affecting the growth of plants (indole acetic acid (IAA), and abscisic acid (ABA)) contents in mung bean seedlings were also evaluated to explore the mechanism of PTW-induced effects in mung bean sprouts growth.

2. Materials and methods

2.1 Materials and Chemicals.

Fresh mung beans used in the experiment were purchased from local market in Xiamen City, Fujian, China. Uniform beans based on shape and size were selected for the experiment and kept at 4 °C until used. Analytical grade chemicals and distilled water were used in the study.

2.2 Plasma Device and PTW Generation.

An atmospheric-pressure plasma jet was used to generate PTW, as shown in Fig. 1. The plasma was ignited by a home-made direct current power source between a tungsten steel tube (1.02 mm and 6.35 mm in inner and outside diameters, respectively) and an aqueous solution surface. A 10-kΩ resistor was connected in series with the tungsten steel electrode to avoid the plasma transfer from glow-like discharge to arc. The feeding gas, such as He, N_2, air, or O_2 was added into the discharge tubes from the top of the device at the flow rate of 20 sccm. A graphite rod (5 mm in diameter) was placed at the bottom of the solution to act as an inert cathode electrode (positive voltage applied to the tungsten steel electrode), the discharge gap was 3 mm, and the discharge current was 30 mA. To refresh the surface liquid, the total 150 ml liquid was circuited by a peristaltic pump at a flow rate of 200 ml/min. With this method, the plasma jet was formed in the vicinity of the end of tungsten steel tube. Sterile distilled water of 150 ml was activated for 30 min to obtain PTW solution which contains a variety of microbicidal active agents (Lukes, Dolezalova, Sisrova, & Clupek, 2014; Zhou et al., 2016a; Zhou et al., 2016b).

2.3 Physicochemical Properties Measurement.

Optical emission spectra (OES) were obtained to identify the major excited reactive species during PTW generation by the N_2, He, air, O_2 plasma, using a SpectraPro-750i monochromator (Acton Research Corporation) with its resolution of 0.5 nm in the wavelength range of 200-800 nm. One end of the fibre optic cable was used to acquire the light signals through a focus lens at a distance approximately 3 cm from the side of the generated plasma. From OES of different gaseous plasmas, the global levels of ROS generated by plasma in the solution could be approximately investigated.

To analyze the composition of the plasma gas effluent, an ozone meter (JA-1000-O3) was used to measure O_3 concentration. A multi-gas detector (RAE Systems, PGM-7800) was applied to detect NO and NO_2 concentration. In addition, the ORP, pH values and temperature of the PTW solution were measured by a multimeter pH and Redox (Mettler-Toledo, Switzerland), and the electrical conductivity was measured by an electric conductivity detector (Yesmylab SX650). The measurements showed that the temperature of the PTW was usually lower than 40 °C. For reactive species in PTW, the NO_2^- concentration was determined by the well-known Griess assay, which is based on the reaction with sulfanilic acid and N-(1-naphthyl)-ethylene diamine hydrochloride via azo sulfanilic acid to a magenta colored azo dye whose absorption at 525 nm can be measured (Jablonowski et al., 2015; Boxhammer et al., 2012). The NO_3^- concentration was also measured via Griess assay, through the conversion to NO_2^- by the use of vanadium(III) chloride (Jablonowski et al., 2015; Boxhammer et al., 2012). The H_2O_2 concentration was measured by Hydrogen Peroxide Assay Kit (Beyotime, Jiangsu, China).

2.4 Treating Seeds with PTW.

100 uniform seeds were placed on the filter cloth in 9 cm petri dishes and 10 ml of N_2-PTW, He-PTW, Air-PTW, and O_2-PTW solution was added into each dish respectively to create germination condition. Meanwhile, the same number of seeds in the control group were also subjected to the same volume of distilled water. After that, these samples were incubated in a

light incubator at the temperature of 25 °C. During the germination and growth, 5 mL of the same type of PTW was used to water the treated mung beans 4 times every day until the mung bean sprouts were harvested.

The effects of PTW generated with different feed gases on seed germination and seedling growth experiments were studied respectively in this research. The germinated seeds in each dish were counted every 3 hours for 7 days, while the total length of mung bean sprouts and the diameter of hypocotyls were measured every 12 hours by a rule with the precision of 0.1 cm and by a Vernier caliper, respectively. Several parameters utilized to describe the statistical characteristics of seeds including germination percentage, germination potential, and germination index were calculated by following formulas (Bormashenko, Grynyov, Bormashenko, & Drori, 2012; Ling et al., 2014):

Germination percentage (%) = $N_t / N_{TS} \times 100$ (1)

Germination potential (%) = $N_{3D} / N_{TS} \times 100$ (2)

Germination index $G_i = \sum \frac{N_{Dn}}{D_n}$ (3)

Vigor index $V_i = G_i \times L$ (4)

List of investigated characteristics:

N_t : Number of total germinated seeds after incubation time of t;

N_{TS} : Number of total seeds per dish;

N_{3D} : Number of total germinated seeds per dish in the first 3 days;

N_{Dn} : Number of germinated seeds on the day n

D_n : Germination day

L : The total length of the seedling.

The morphological measurements of mung bean sprouts were performed at the intervals of 12 h after germination began. 20 pieces of mung bean sprouts of each treatment were measured and the average length and diameter was calculated. Moreover, scanning electron microscopy (SEM, Hitachi 4800, under vacuum at 10–15 kV) was used to investigate the effect of PTW treatment on the morphological characteristics of mung bean seed coat surface (Koga et al., 2015; Bormashenko et al., 2012).

2.5 Microbiological Analysis.

The total bacterial numbers on the mung bean sprouts produced by different PTW were counted according to the method described by Koide Shoji, with modifications (Koide, Takeda, Shi, Shono, & Atungulu, 2009). After germination (Day 7), all the collected mung bean sprouts were weighed into 200 mL of sterile physiological saline solution. The flask was shaken vigorously by hand to remove the microbial cells from the surface of sprouts, and then 1 mL of the solution was aspirated aseptically and diluted to 10 mL with sterile physiological saline solution. For the enumeration of total bacterial count, 1 mL of the mixture was serially diluted (1:10) in the sterile saline solution, and 100 μl of the diluted solutions were spread uniformly in triplicate on LB plate, and then incubated at 37 °C for a subsequent CFU counting of bacteria. The microbial count was expressed as log colony forming units per gram (log CFU/g). The sterilization ability of PTW was evaluated by the Log Reduction, which was calculated by the following formula:

$$\text{Log Reduction} = \text{Log}\ (CFU_{control} / CFU_{PTW\text{-}treated}) \quad (5)$$

2.6 Determination of SOD activity, MDA, IAA and ABA contents in mung bean sprouts.

The determination of SOD activity in the seedlings was based on the nitroblue tetrazolium method (Xu, Guo, Bai, Shang, & Wang, 2009). In brief, mung bean seedlings (1 g) mixed with quartz sands (0.4 g) and 5 ml of 100 mM phosphate buffer (PBS, 1.3 mM NaH_2PO4, 8.7 mM Na_2HPO_4, 0.14 M NaCl, pH 7.4) containing 1 mM EDTA and 5% (w/v) polyvinylpyrrolidone (PVP) were ground on ice. The supernatant got after centrifugation at 10 000 rpm for 30 min at 4 °C, was used for SOD analysis. 15 μL of the supernatant (or distilled water acting as the control group) was added into a test tube and mixed with 2.55 ml of 100 mM phosphate buffer (pH 7.8), 75 μl of 55 mM methionine, 300 μl of 0.75 mM nitroblue tetrazolium (NBT) and 60 μl of 0.1 mM riboflavin. The test tubes were then irradiated for 10 min under a set of fluorescent light tubes of 40 μmol m^{-2} s^{-1}. The absorbance of the irradiated and non-irradiated (blank group) solutions was determined at 560 nm (1.5 mL). One unit of SOD activity was defined as the amount of enzyme that would inhibit 50% of NBT photoreduction. The total activity of SOD was calculated based on the following expression:

$$\text{SOD total activity (U/mg FW)} = \frac{(A_0 - A_S) \times V_T}{0.5 \times A_0 \times W \times V_1} \quad (6)$$

A_0 : The absorbance of the control one at 560 nm;

A_S : The absorbance of the samples at 560 nm;

V_T : The total volume of the samples in the test tubes (mL);

W : The weight of the mung bean seedlings used (mg);

V_1 : The volume of the supernatant used for each measurement (mL).

The content of malonaldehyde (MDA) was measured based on the method described by Dhindsa et al. (Dhindsa, Plumb-Dhindsa, & Thorpe, 1981) with some modifications. Mung bean seedlings (0.15 g) were homogenized by grinding in 4 ml of 10% TCA before the samples were subjected to centrifugation at 10 000 rpm for 15 min. The supernatant (1 ml) was mixed with 1 ml of 0.6% thiobarbituric acid, heated at 95 °C for 30 min and then was quickly cooled down on ice. After centrifugation at 10 000 rpm for 10 min, the specific absorbance of the product and the non-specific, background absorbance was read at 450, 532, and 600 nm, respectively. The detailed process was similar to that research reported in the reference (Liu, Song, Zhou, & Niu, 2014). The equation (7) was used to determinate the content of MDA in the raw mung bean seedling samples.

$$\text{MDA content (nmol/mg FW)} = 8 \times \frac{6.45\times(A_{532}-A_{600})-0.56\times A_{450}}{1000\times W} \qquad (7)$$

here, A_{450}, A_{532} and A_{600} represent the absorbance read at 450, 532, and 600 nm; and W is the weight of raw mung bean seedlings used.

Phytohormone extraction and purification were modified from Yang et al (Yang, Zhang, Wang, Zhu, & Wang, 2001). About 0.500 g mung bean seedlings was homogenized in pre-chilled 80% methanol on ice in weak light condition, with about 0.1 g polyvinylpyrrolidone (PVP) added as antioxidant. Homogenant was centrifuged at 5000 rpm for 10 min at 48 °C, debris was cleaned with pre-chilled 80% methanol and centrifuged. Supernatant was combined and purified with C18 columns (C18 Sep-Park Cartridge, Waters Corp., Millford, MA). For IAA analysis, sample was dried under N_2 gas and then dissolved in 200 ml methanol. Two hundred microliter diazomethane was added for methylation, followed by drying under N_2 gas and dissolved in 300 ml phosphate buffer solution for enzyme-linked immunosorbent assay (ELISA). For ABA analysis, ELISA method was also adopted, and the detailed procedure was similar to that reported in the reference (Ross, Elder, Mcwha, Pearce, & Pharis, 1987).

2.7 Statistical Analysis.

For each treatment, data were obtained from at least 3 independent replicate trials ($n \geq 3$). The mean ± standard deviation (SD) was used to express the value for each experiment. The significance of differences was statistically analyzed using one-way analysis of variance (ANOVA) with a confidence level at $P<0.05$. All statistical analysis was performed using Microsoft Excel 2016 (Microsoft Corporation, USA).

3. Results and discussion

3.1 Major Excited Reactive Species in gas-phase plasmas.

OES spectra was used to investigate the main excited active species generated by N_2, He, air, and O_2 plasma ranging from 200 to 800 nm respectively. As shown in Figure 2, the optical emissions from the N_2 second positive system ($C^3\Pi \rightarrow B^3\Pi$) at 316, 337, 357, 380, and 405 nm (Sakamoto, Matsuura, & Akatsuka, 2007), and the first negative system of N_2^+ ($B^2\Sigma_u^+ \rightarrow X^2\Sigma_g^+$) at 391.4 nm were visible for all plasma generated under N_2, He, air, and O_2 gaseous discharges (Sarani, Nikiforov, & Leys, 2010). The N_2 ($B^3\Pi$ and $C^3\Pi$), and N_2^+ ($X^2\Sigma_g^+$) states are mainly formed by the electron impact excitation from the molecular ground state N_2 ($X^1\Sigma_g^+$) (Liu, Niu, & Yu, 2011). The OH emissions from the transition of $A^2\Sigma^+ \rightarrow X^2\Pi$ ($\Delta v=0$) at 309 nm were also observable for the N_2, He, air, and O_2 plasma jets. The O_2 plasma jet showed the strongest OH emission while the opposite was the one for N_2 plasma jet. Water vapor molecules might diffuse into the plasma, then OH radicals could be formed by electronic impact dissociation ($H_2O + e^- \rightarrow H + OH + e^-$) (Zhou et al., 2015). While in He plasma, OH radicals was generated by the penning process of excited He* and water vapor ($He^* + H_2O \rightarrow He + H + OH$) (Zhou et al., 2015).

The O emission at 777 nm, corresponding to the oxygen atom transition of O ($2s^2 2p^3 3s$-$2s^2 sp^3 3p$), was visible for air and O_2 plasma. The energetic collisions of electrons with O_2 molecules ($O_2 + e^- \rightarrow 2O + e^-$) were responsible for the formation of O radicals. The recombination process of O and H radicals ($O + H + M \rightarrow OH + M$) could contribute to the generation of OH radicals in the air and O_2 plasma (Zhang et al., 2016). The results of OES indicated that excited OH radicals, atomic oxygen and nitrogen were generated in the gaseous plasma, and could transfer into the liquid and then could be easily converted to other ROS and RNS in the solution, such as hydrogen peroxide (H_2O_2), nitrite (NO_2^-) and nitrate (NO_3^-), which will be analyzed and discussed later.

The O_3, NO_2 and NO concentration in different plasma gas effluent were also investigated. The air plasma showed the O_3 and NO_2 concentration for 83.75 and 138.51 ppm, and the O_2 plasma showed the O_3 and NO_2 concentration for 90.92 and 51.69 ppm, while the N_2 and He plasma displayed only at 3.10 and 23.56 ppm, and 4.03 and 7.41 ppm respectively. In addition, the NO concentration in these plasma effluents was only 1~2 ppm (below the detection limit), this might because NO converts quickly to NO_2 via reacting with O_2.

3.2 Physicochemical Properties and Bactericidal Effects of PTW

The OPR, conductivity, pH values and the concentration of plasma-generated long-lived chemical species, including NO_3^-, NO_2^- and H_2O_2, in N_2-PTW, He-PTW, O_2-PTW and Air-PTW were measured and the total bacterial counts on the surface of mung bean sprouts were enumerated. As shown in Table 1, compared to control (251 mV), the ORP of O_2-PTW and Air-PTW markedly increased to 610 and 650 mV respectively, while the He-PTW and N_2-PTW showed the relatively lower ORP values (298 and 304 mV respectively). This result indicates that a large amount of ROS was generated in PTW, which has been considered to play a critical role in the PTW inactivation process. Electrical conductivity is also an important indicator to reflect the level of active ions that existed in PTW. Results showed that the electrical conductivity of water increased dramatically from 3.05 to 143.24 μS/cm after 10 min of air plasma activation, and 143.33, 145.68 and 141.33 μS/cm for N_2-PTW, He-PTW and O_2-PTW, respectively. This is mainly because various ROS and other reactive chemical species derived from chemical reactions between water molecules and plasma electrons (Maeda, Igura, Shimoda, & Hayakawa, 2003). Water treated by atmospheric-pressure air and N_2 plasma jets resulted in a slight decrease in the pH value of the solution. This is attributed to the effects of nitric and nitrate acids produced from the reaction of H_2O molecules with NO_x species (Zhou et al., 2016a; Zhou et al., 2015). On the other hand, the pH values of the solutions treated by O_2 and He plasma increased only slightly, mainly because energetic collisions of electrons with water vapor molecules would result in the formation of OH species in water and thus lead to an increase in the pH value.

Moreover, several plasma-generated long-lived chemical species in PTW, such as H_2O_2, NO_3^- and NO_2^- were also listed in Table 1. Among these species, H_2O_2 formation is mainly attributed to the high electron density, energy of the plasma and long lifetime of the excited species that facilitate the energy transfer between the excited plasma species and water molecules ($*e^- + H_2O \rightarrow \bullet H + \bullet OH + e^-$, $\bullet OH + \bullet OH \rightarrow H_2O_2$). NO_2^- and NO_3^- are formed in plasma-treated water through the dissolution of nitrogen oxides formed in the plasma by gas-phase reactions of dissociated N_2 and O_2 or H_2O. Clearly, the H_2O_2 concentration in O_2-PTW, Air-PTW reached approximately 1.76 and 1.29 mM after O_2 and air plasma treatment for 30 min, while the one in N_2 and He-PTW displayed at the concentration of 0.56 and 0.85 mM respectively. For plasma induced nitrogen-containing species, Air-PTW and N_2-PTW show higher concentration of NO_3^- and NO_2^- (2.43 mM and 0.54 mM for Air-PTW, 1.36 mM and 0.29 mM for N_2-PTW, respectively), when compared with He-PTW and O_2-PTW. In addition, the microorganism counts of sprouts were found to be strongly dependent on feed gases used for PTW generation, which can be concluded from Table 1. Microorganism counts of sprouts produced only by water (control) reached as high as 6.21 log CFU/g, while Log Reduction of the sample treated by Air, O_2, He and N_2-PTW was 5.17, 4.29, 2.80 and 2.04 respectively. These four kinds of PTW treatments all showed significant effects on microorganism sterilization to mung bean sprouts. Compared to O_2, He and N_2-PTW treatments, Air-PTW treatments could significantly enhance the microbial inhibiting ability of PTW, resulting in plate count significant reductions. This may be attributed to the fact that H_2O_2 and NO_2^- radicals generated in Air-PTW may form more oxidative species such as peroxynitrous acid (ONOOH) and peroxynitric acid (O_2NOOH) known to have strong bactericidal effects under the acidic condition (Shen et al., 2016).

3.3 Effect of PTW on Seed Germination and Seedling Growth of Mung Bean.

Figure 3 and Table 2 show the effects of different types of PTW on seed germination and seedling growth of mung bean. All types of PTW generation were performed with a discharge current of 30 mA. As the data suggested, the O_2 and Air-PTW treatments were more efficient in accelerating and enhancing seed germination, for example, compared with the control group, Air-PTW treatment was capable of shortening the germination time needed from 72 h to 36 h, as well as improving the final germination percentage by around 6%, which could be attributed to the relatively high density of reactive oxygen species generated in air and O_2 plasma (Zhou et al., 2015). To be specific, after 12 h of incubation, the germination percentage of the groups treated by Air-PTW and O_2-PTW reached 36.33% and 11.67% respectively, while the germinated seeds in the other three groups (control, N_2-PTW and He-PTW) were almost not witnessed. The germination percentage of Air-PTW and O_2-PTW treated samples kept soaring to 93.67% and 83.67% respectively at the incubation time of 24 h, significantly higher than that of He-PTW (51.33%), N_2-PTW (42.33%) and the control (29.67%). Germination percentage achieved after incubation in the first 3 days usually named as germination potential is often used to estimate the germination ability of seeds. Clearly seen from Table 2, only Air-PTW and O_2-PTW treatments possessed the ability to increase the germination potential of mung bean by around 6% and 3.6%.

In addition, germination index and seedling growth (total plant length, diameter of hypocotyl and vigor index) are also the most significant parameters to show the biological vigor of seeds (Ling et al., 2014). Compared with the control, O_2 and Air-PTW treatments remarkably increased the germination index by 31.39 % and 37.72% respectively, but there was witnessed only slight improvement by N_2 or He-PTW treatment (about 10%). With the increase of incubation time to 7 days, the plant length of Air-PTW treated seeds reached 12.01 cm, increased by around 100% compared with the control ones, showing noteworthy advantage to other treatments. O_2-PTW also showed acceptable improvement in seedling length (8.07 cm), while N_2 and He-PTW treated samples displayed only marginally higher plant lengths than those in the control group. Moreover, the vigor index was increased by 36.62% and 41.78%, compared to the control, by the N_2 and He plasma treatments respectively, while the corresponding value for O_2 and air treatment was estimated to be approximately two and three times than that for the control. However, one thing should be pointed out that the diameter of hypocotyl in all the five groups was around 1.9 mm, which meant PTW treatment didn't showed significant effects on the diameter of hypocotyl. The maximum germination potential, germination rate, germination index and vigor index of seeds were obtained from the Air-PTW treatment, indicating that Air-PTW treatment was the optimal approach to improve the germination of mung bean seeds.

3.4 SEM Images of the Surface of Mung Bean Seeds

SEM (Scanning electron micrograph) images of seed coat surface were investigated with respect to their morphological characteristics, as shown in Figure 4. It can be noticed that surface structure of seeds was changed sharply as a result of PTW treatments. Figure 5(a) indicates that the surface sculpture of bean seeds soaked in distilled water for 4 hours had an irregular rhoptry-shaped pattern with the size varying from 1.0 to 3.0 μm. With N_2 and He - PTW treatment for 4 hours, the seed coat of mung bean chapped slightly, while O_2 and Air-PTW treated seeds had an eroded surface, with no significant ridges. These results indicate that high concentration of ROS induced by O_2 and air discharge in water might contribute to the chapping of seed coat, which improved its absorption of water and nutrients, a condition that could enhance the germination rate of mung bean and promote the growth of hypocotyl and radicle (Dobrin, Magureanu, Mandache, & Ionita, 2015; Stolárik et al., 2015).

3.6 Effects of PTW on the SOD activities and MDA contents in mung bean seedlings

It is known that a balance among the scavenging enzymes is required to detoxify ROS in the cell. SODs are the major O_2^- scavengers and provide a first line of defense against the cellular injury by environmental stresses (Gratão, Polle, Lea, & Azevedo, 2005). SOD activities in the mung bean seedlings were markedly raised in all PTW treatments compared with the control and the highest went for Air-PTW treated samples (Figure 6(a)). Air and O_2-PTW treated samples significantly increased the SOD activity by 77.27% and 63.63% respectively, compared to the control seedlings. Wu et. al. suggested that plasma treatment could play an important role in regulating water balance by modulating antioxidant enzymes (Wu, Chi, Bian, & Xu, 2007), and Henselová Ľudmila et al. pointed out that plasma treatment stimulated SOD and POD activities in tomato seedlings (Henselová, Slováková, Martinka, &

Zahoranová, 2012). Results in this study implicated that the Air-PTW plays an important role to reduce oxidative damage and helps to maintain normal physiological metabolic activities, leading to improved mung bean seedling growth.

MDA is the product of membrane peroxidation and has been used as a direct indicator of lipid peroxidation and membrane damage (Liu, Niu, & Yu, 2013). Figure 6(b) shows the effects of N_2, He, Air and O_2-PTW treatments on MDA contents in mung bean seedlings. Clearly, MDA contents in the mung bean seedlings were remarkably reduced after PTW treatments. The MDA content in Air-PTW treated samples was significantly reduced by 32.50% and that was reduced by 22.50% in O_2-PTW treated samples. The present study demonstrated that Air-PTW treatment could reduce membrane lipid peroxidation damage by increasing antioxidant enzyme activities, thus significantly reducing the accumulation of MDA. On the other hand, it was detected that air plasma generated RNS radicals (nitrogen oxide (NO_X) molecules, HNO_X) 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, as shown in our previous study (Zhou et al, 2016a), 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-PTW had a lower rate of electrolyte leakage, making it possible for the seeds to maintain relatively high activity (Liu et al., 2013). The higher root activity of mung bean sprouts further contributed to the growth of the sprouts.

3.7 Effects of PTW on the phytohormone contents in mung bean sprouts.

Indole acetic acid (IAA) has a wide range of physiological functions, which could regulate multiple proceedings, including cell division, elongation and differentiation, even the growth, maturity and aging of the vegetative and reproductive organs. The most obvious physiological effect of IAA is to promote cell elongation growth. The effect of different types of PTW on the IAA content in mung bean sprouts is shown in Figure 6(a). From the first day to the second day, the IAA content of each treatment group had a dramatic increase and reached a maximum value on the second day after germination, and then that stayed at a basically dynamic equilibrium state on the 2nd to 5th day. In addition, the results show that the IAA content of Air-PTW treatment group was significantly higher than the other groups, also indicating that Air-PTW could significantly promote seed germination and seedling growth.

Abscisic acid (ABA), a hormone that inhibits the growth of plants, acts obstructively to the cellular fission and elongation and the growths of coleoptile, sprays, roots, hypocotyl and other apparatus, thus helps to maintain the stable dormancy status of various sorts of xylophyta and seeds. As is shown in Figure 6(b), the contents of ABA, of which treated by Air-PTW, O_2-PTW and N_2-PTW respectively, kept reducing during the whole germination processes. While that of He-PTW witnessed slow decrease in the first 3 days, then increased significantly in day 4. Moreover, the content of ABA which generated by the mungbean sprout with Air-PTW presented a lower level than those of other samples. Wang M. et al. studied the effects of chemicals that break dormancy on the content of ABA during the growth of barley (Wang et al., 1998). The obtained medicals were divided to two sorts: dormancy breaking substance (I), such as gibberellin, alcohol, H_2O_2, nitrate, ortho-hydroxybenzoic acid, etc, resulting in the decrease of endogenous ABA; The other is a sort of substance (II) which weakens the influences of ABA, but without effects on the content of ABA, including chitosan, sulphuric acid, sodium azide, acetic acid and so on. The substance I influences the activities of enzymes that associates with the synthesis or degradation of ABA. Previous research has shown that reactive oxygen species can accelerate the degradation of

ABA (Sandberg, Crozier, & Ernstsen, 1987). A variety of ROS could be generated in the Air-PTW, such as H_2O_2 and ·OH, thus reducing the content of ABA in mung bean sprouts with the Air-PTW treatment.

4. Conclusion

In this study, one home-made plasma jet was designed and used to produce PTW with four commonly processing gases. Compared to the O_2, N_2 and He-PTW treatment, the Air-PTW displayed the highest oxidation reduction potential (650 mV), the lowest pH (3.5) and the highest plasma-generated long-lived RONS (up to 4.26 mM), which are attributed to the efficiency of Air-PTW in decontamination (with a Log Reduction of 5.17), seed germination (germination index reaching 95.50%) and seedling growth (vigor index increased to 1146.64) of mung bean. Measurements also showed that the Air-PTW treated mung bean seedlings possessed the highest SOD activity and IAA content, lowest MDA and ABA contents, indicating that it was capable of reducing oxidative damage, increasing antioxidant enzyme activities, and promoting seed germination and seedling growth. Our results suggest that Air-PTW has the potential to enhance the seed germination and seedling growth of mung bean and reduce the level of microbial contamination of mung bean sprouts.

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Figure captions

Figure 1. (a) The schematic diagram of the experimental setup used in this study, and (b) a photograph of the plasma-liquid interactions producing PTW.

Figure 2. Typical OES spectra of the N_2, He, Air, and O_2 plasma generated with a discharge current of 30 mA. a.u., arbitrary units.

Figure 3. The germination percentage of mung bean seeds treated with He-PTW, N_2-PTW, O_2-PTW and Air-PTW as a function of incubation time.

Figure 4. SEM images of mung bean seeds' surface (a) control sample soaked in distilled water, (b) N_2-PTW treatment, (c) He-PTW treatment, (d) O_2-PTW treatment, and (e) Air-PTW treatment. All treatments are performed for 4 hours. Scale bar is 10 μm.

Figure 5. Effects of different on the SOD activities (a) and MDA contents (b) in mung bean seedlings.

Figure 6. Effects of different types of PTW treatment on the contents of indole acetic acid content (IAA) (a) and abscisic acid (ABA) (b) in mung bean seedlings.

Tables

Table 1. Physicochemical properties (ORP, conductivity, pH and concentration of long-lived RNOS (NO_3-, NO_2- and H_2O_2)) in N_2-PTW, He-PTW, O_2-PTW, Air-PTW and microorganism count statistic results. Results labeled with different letters across the treatments represent significant differences.

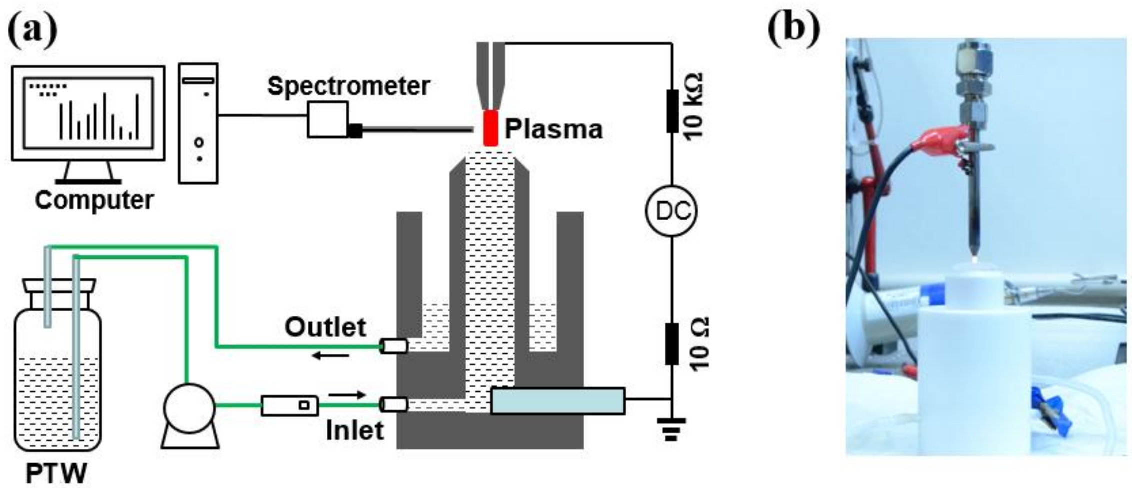
Treatment	ORP (mV)	Conductivity (μS/cm)	pH value	NO_3^- (mM)	NO_2^- (mM)	H_2O_2 (mM)	Log Reduction
Control	251±2.49d	3.05±0.10b	7.0±0.08bc	--	--	--	0.00±0.05e
N_2-PTW	304±3.68c	143.4±1.38a	6.5±0.24c	1.36±0.04c	0.29±0.02c	0.56±0.03d	2.04±0.13d
He-PTW	298±5.31c	144.8±3.41a	7.5±0.22b	0.12±0.04b	0.03b	0.85±0.03c	2.80±0.11c
O_2-PTW	610±3.56b	141.0±1.63a	7.4±0.08b	0.13±0.02b	0.05b	1.76±0.05b	4.29±0.10b
Air-PTW	650±5.56a	143.2±3.41a	3.5±0.34a	2.43±0.04a	0.54±0.04a	1.29±0.04a	5.17±0.07a

-- meant that the result is below the detection limit.

Table 2. Effects of N_2, He, O_2 and Air-PTW on seed germination and seedling growth of mung bean. Results labeled with different letters across the treatments represent significant differences.

Treatment	Germination potential (%)	Germination index (%)	Total plant length (cm)	Vigor index	Diameter of hypocotyl (mm)
Control	91.00 ± 0.82c	57.78± 0.45d	6.05±0.14c	349.76 ± 10.48e	1.94 ± 0.07a
N_2-PTW	90.33 ± 0.94c	68.56± 0.80c	6.97±0.21c	477.84 ± 13.54d	1.97 ± 0.12a
He-PTW	91.00 ± 1.41c	69.94 ± 0.64c	7.09±0.09c	495.90 ± 7.10c	1.91 ± 0.04a
O_2-PTW	94.67 ± 0.47b	89.17 ± 1.25b	8.07±0.13b	719.28 ± 8.55b	1.88 ± 0.08a
Air-PTW	97.33 ± 0.47a	95.50 ± 0.41a	12.01±0.19a	1146.64 ± 19.65a	1.98 ± 0.04a

Figures



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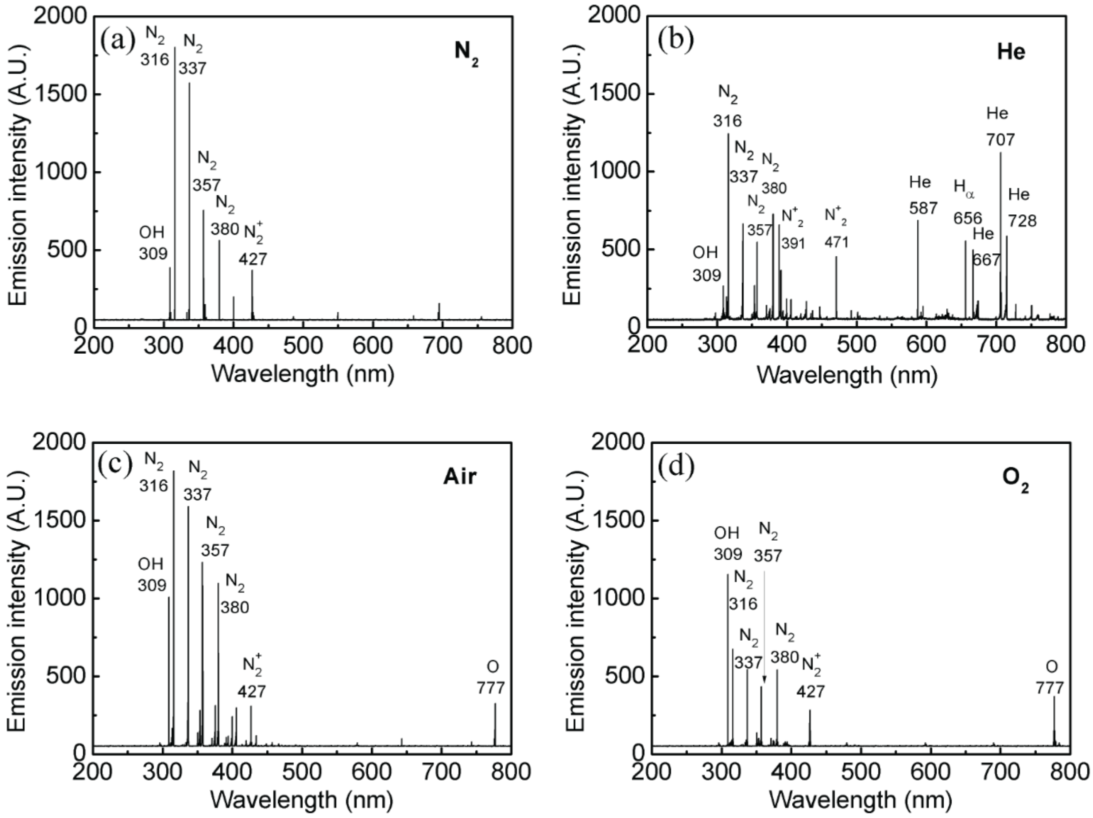

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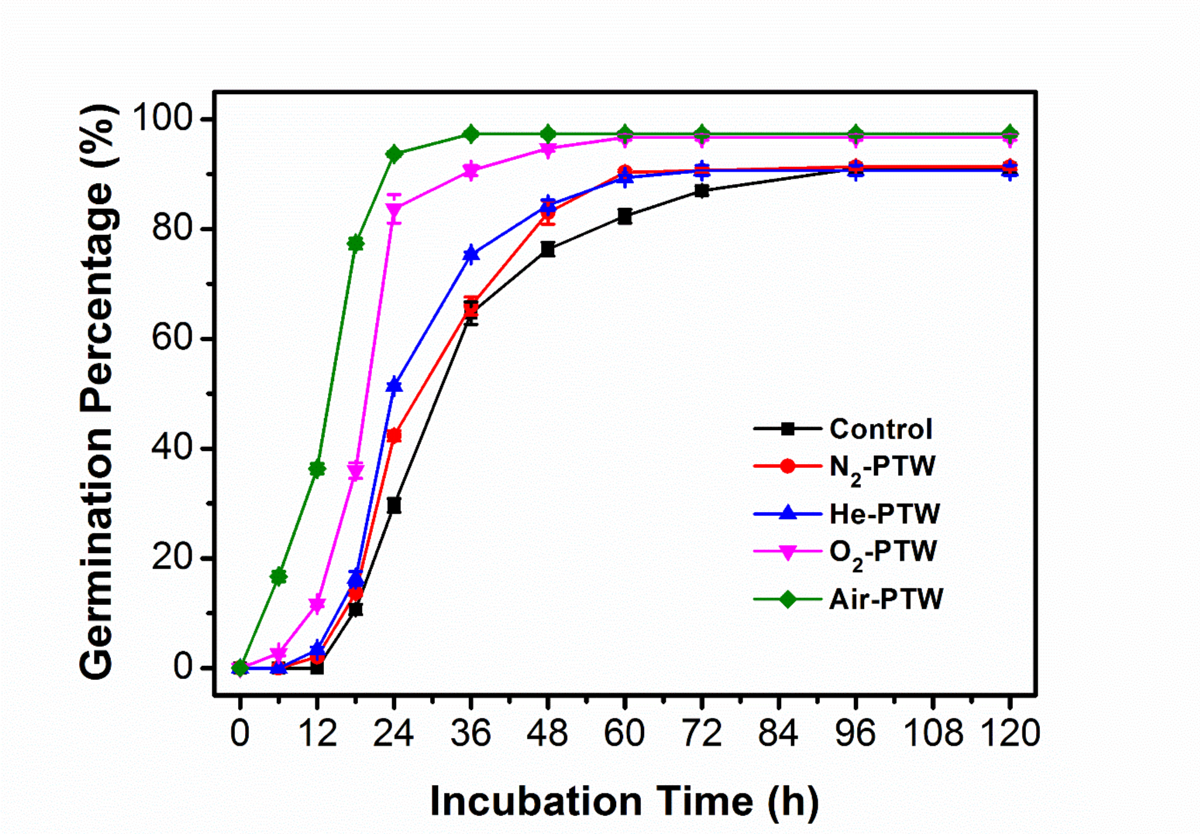

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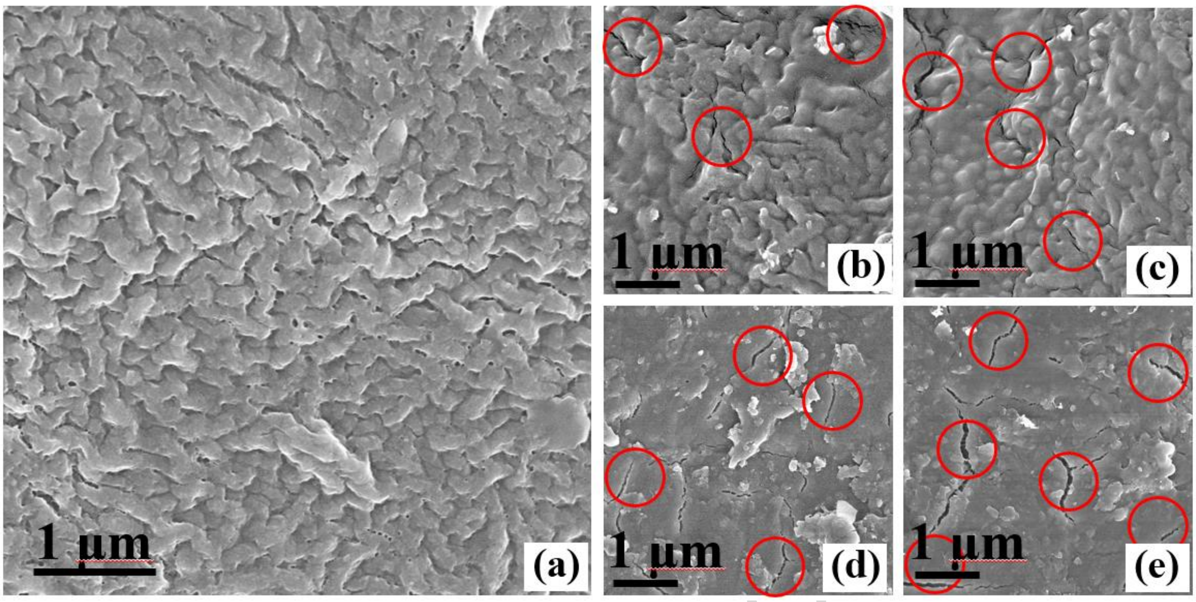

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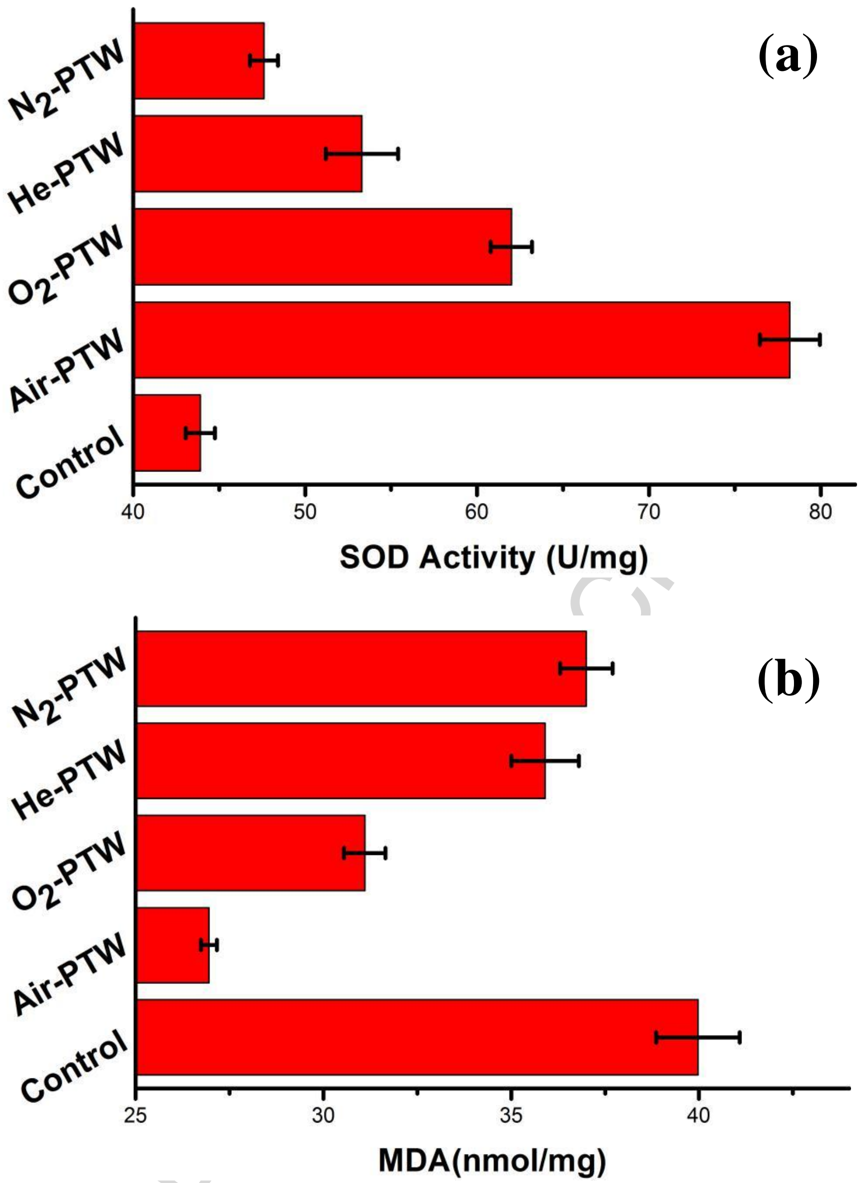

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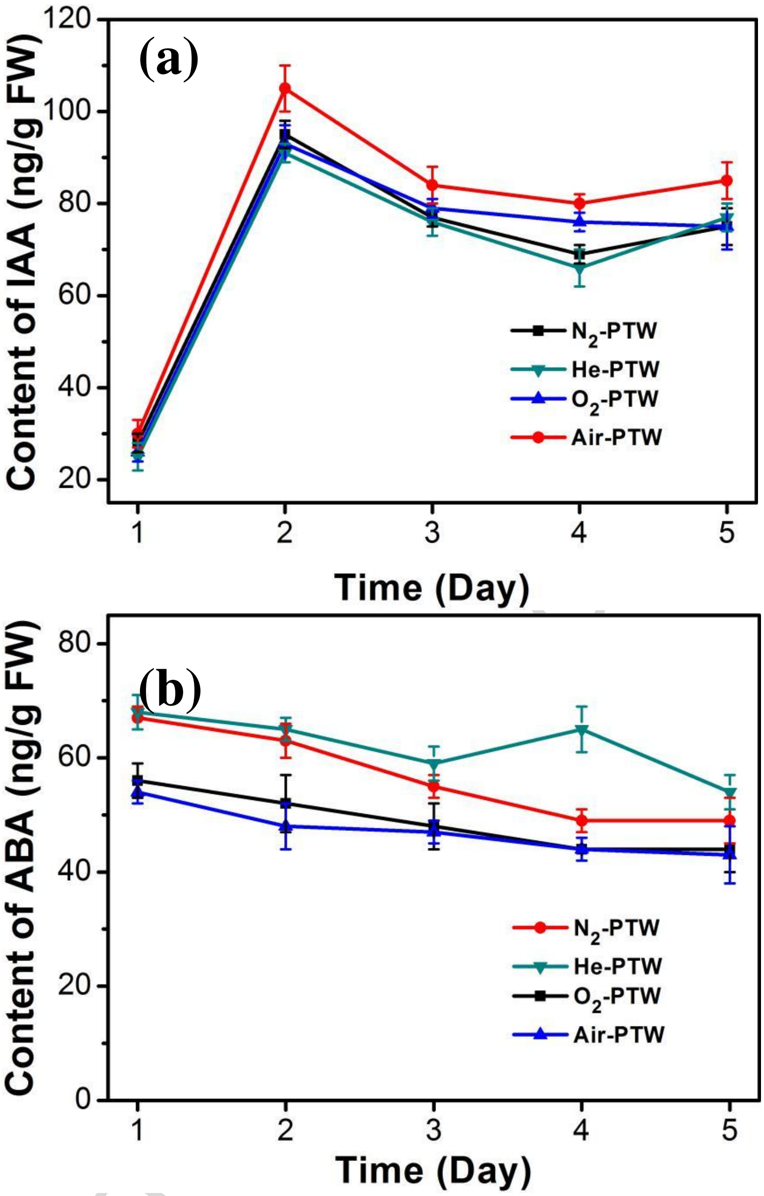
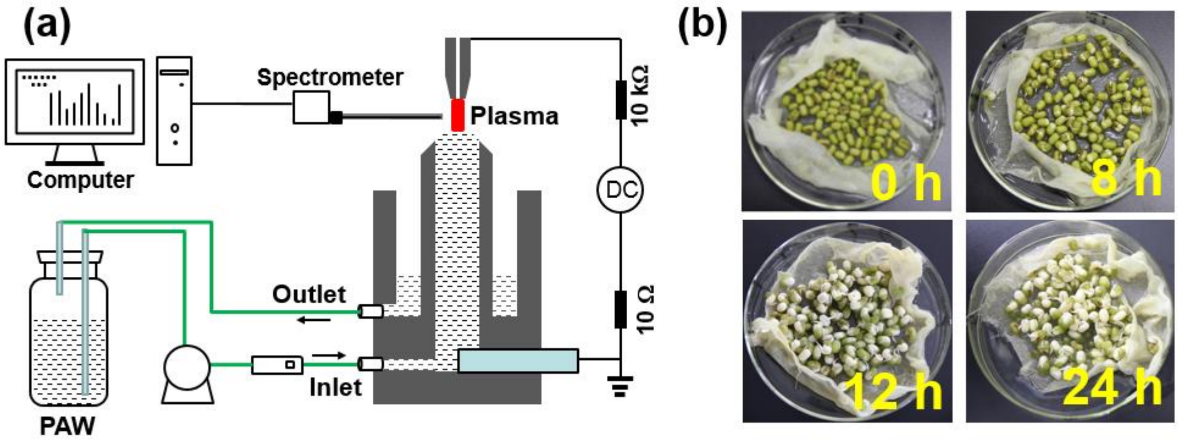


Figure 6. Effects of different types of PTW treatment on the contents of indole acetic acid content (IAA) (a) and abscisic acid (ABA) (b) in mung bean seedlings.

Graphical Abstract



Highlights

- Plasma-treated water (PTW) was applied into mung bean germination for the first time.
- The effects of PTW on seed germination, seedling growth, and sprout sterilization were studied.
- Air-PTW has the potential for use as a green and effective mean for improving seed germination.