



RESEARCH ARTICLE

Seed vigor of soybean treated by corona discharge plasma

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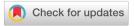


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Abstract

There is a huge gap between the output and demand of soybean in China. How to improve the seed vigor of soybean has always been a research focus. Low temperature plasma (LTP) is a new green technology, which is widely used in crop seed treatment. Corona plasma is a typical discharge mode of plasma, which can affect the vigor of seeds. The effect of different discharge power on the soybean seed vigor by plasma treatment was experimentally investigated. Plasma discharge characteristic wavelength and spatial distribution were analysed. It shows that the corona discharge spectrum mainly exhibits the strong ultraviolet radiation and 90% of the spectral intensity focused in the center of discharge region. Water absorption and germination index of seeds and the fresh weight of seedlings were used to characterize the specific effects caused by different plasma powers. The results show that plasma treatment has a significant effect on the early stage of germination and can significantly affect the soybean seed vigor and growth. Overdose treatment will cause inhibiting effect. This study provides an experimental basis for the practical agriculture application of corona plasma seed treatment.

Keywords

Plasma treatment; soybean; seed vigor; spectrum

Introduction

Soybeans are an indispensable crop rich in plant protein and have been cultivated in China for more than five thousand years (1). Improving the yield and quality of soybean has always been a key concern. Seed quality is generally judged by seed vigor, which represents the comprehensive abilities of seed germination, seedling growth, and the plant's ability to resist stress (2). Over the past decades, researches have explored various physical, chemical and other intervention strategies to stimulate seed germination and sprouting, such as pesticides, electrolysis of oxidized water, ultrasound, electromagnetic fields, and plasma (3, 4). Low temperature plasma (LTP) is a green technology that can be employed in agriculture. Treating seeds with LTP before sowing has positive biological effect on plants (5). Numerous studies have demonstrated that LTP treatment significantly enhances plant growth and yield (6, 7). During the treatment process, LTP effectively eliminates pathogenic bacteria on the seed surface without damaging the seeds (8). In a study involving lentil seeds, LTP was utilized as a seed decontamination technology for germination production. The results indicated that

LTP could effectively reduce the activity of *E. coli* and *Salmonella typhimurium* on the seed surface, while preserving the germination characteristics of seeds (9). Simultaneously, plasma discharge generates active species such as electrons, ions, excited atoms and molecules, free radicals, among others. These active species can improve the seed respiration and water absorption, enhancing the activity of enzymes within seed, including amylase, lipase, proteinase activity, and antioxidant enzyme activity. Therefore, this process effectively promotes seed germination (10–12).

Corona discharge, a type of local self-sustained discharge in a gaseous medium within a non-uniform electric field, is a typical LTP. This phenomenon occurs typically under atmospheric pressure in region of strong electric fields, near sharp edges, points, or thin wires (13). It is a chemical-physical treatment technology known for its simple devices, evident biological effects, and environmental friendliness. The biological impact of corona discharge results from two primary factors: the nonuniform electric field and active substances generated by plasma discharge. Previous studies (14, 15) have demonstrated that corona discharge treatment significantly alters the structure and properties of seed coat. It induces strong corrugation, creates pores and surface defects, enhances seed hydrophilicity, and promotes seed germination. The fundamental impact of corona plasma treatment on plant growth lies in its effect on seed vigor. While some studies have explored the influence of corona plasma on seeds or plant growth and yield, several aspects still warrant further investigation. For example, understanding the characteristics and spatial distribution of the corona discharge in the treatment of seeds. Additionally, exploring how corona discharge parameters affect the effectiveness of seed treatment is essential. Moreover, determining the specific stage of germination at which plasma action has a more significant effect remains a pertinent area for research.

This paper mainly conducted the experimental study on the vigor of soybean seeds treated in a corona plasma system through adjusting plasma discharge power, making the analysis on discharge spectrum and performing the soybean planting experiments to explore the effects of plasma power on seed vigor and find the optimized plasma treatment condition.

Materials and Methods

Plant material

In this experiment, the soybean with the variety of Zhonghuang 37 was selected for plasma treatment. This soybean variety is suitable for summer planting in northern regions of China, such as Beijing, Hebei and Shandong provinces.

Characteristics of plasma source

Fig. 1 shows the corona discharge equipment used in the soybean processing experiment, comprising a corona plasma generator, a mobile chassis, a control panel, and an exhaust system. Three ceramic square electrode rods, each measuring 600 mm in length, are arranged in parallel to create an effective discharge area in 600 × 300 mm². An exhaust pipe with a diameter of 150 mm is installed on the top of the plasma generator. In Fig. 1 (a), the schematic diagram provides a side view of the soybean treatment setup. The orange squares represent the three groups of discharge electrodes, and the yellow circles represent the seeds passing through the discharge area. Fig. 1 (b) illustrates the right view of the electrode connections, with the top array being high voltage and the bottom array grounded. The control panel is used to adjust the discharge power. Fig. 1 (c), the top view shows the relative orientation of 45° between the optical fiber and the electrode arrays. For spectral analysis, a fiber spectrometer PG2000-Pro was used to detect the discharge spectrum within the wavelength range of 360-1100 nm.

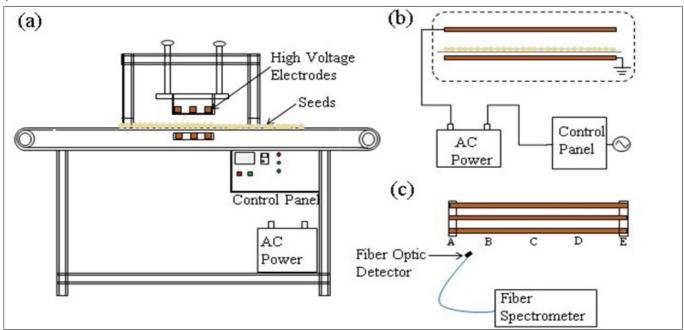


Fig. 1. Schematic diagram of plasma discharge setup: (a) side structure, (b) right view of electrode connection, (c) the optical fiber orientation from the top view.

Seeds treatment

Soybean seeds with smooth surfaces and uniform sizes were selected as experimental samples for plasma treatment. The vigor of plasma-treated seeds depends on various plasma treatment parameters, such as discharge power, treatment time, gas, electrode shape, etc. In this experiment the soybean plasma treatment parameters are outlined in Table 1. The constant included a discharge area of 600 × 300 mm², a treatment time of 4.5 s, discharge gas consisting of air, and a total of 600 treated seeds. The only variable was the discharge power, ranging from 0-6 kW. Based on the treated discharge power, the experimental groups were divided into four groups: group CK (without plasma treatment), and the three plasma treated groups of P2, P4 and P6 with discharge power of 2, 4 and 6 kW, respectively. The paper will investigate the soybean seed vigor in these four groups.

Table 1. Plasma treatment parameters.

| Group | Power (kW) | Time (s) | Discharge area (mm²) | The dis- charge gas | Number of soybean seeds |
|-------|---------------|-------------|----------------------------|---------------------------|-------------------------------|
| СК | 0 | 0 | 0 | | |
| P2 | 2 | | | Air | 600 |
| P4 | 4 | 4.5 | 600 × 300 | | |
| P6 | 6 | | | | |

Seedling characteristics

Following treatment with the aforementioned plasma discharge parameters, a germination test was conducted on the four groups of soybean seed samples under the same place, temperature and humidity conditions (Beijing, China, 25 °C, 45%-50% RH). The growth data of seeds and seedlings were recorded at regular intervals. Prior to germination, proper soaking of soybean seeds is necessary. In our experiment, after the soybean seeds were treated with different plasma powers, they were soaked in a temperature-controlled water tank at 25°C. The water absorption of seeds was measured every 2 hr, and the total water absorption within 24 hr was calculated. Seed vigor was assessed from the onset of soaking until the end of the 8th day. The number of germinated seeds was recorded daily. On the 8th day of germination, the fresh weight of seedlings was measured, and the germination index (GI) and vigor index (VI) (16) were calculated using the following formulas:

$$GI = \sum Gt/Dt$$
 (Eqn. 1)

where Dt is the number of germination days and Gt is the number of germination seeds per day corresponding to Dt.

$$VI = GI \times S$$
 (Eqn. 2)

where S is the average fresh weight (g) of a single plant seedling.

Results and discussion

Discharge voltage measurements

During the soybean treatment process, plasma discharge at different power levels was observed. Fig. 2 displays the plasma discharge images at (a) 2 kW, (b) 4 kW and (c) 6 kW, illustrating the filamentary discharge modes characteristics of corona discharges. The intensity of the discharge in the middle region is stronger than at both ends, and both the discharge area and intensity increase with higher discharge power. The specific relationship between plasma discharge spectrum intensity and position under corona discharge power can be measured through spectroscopy.

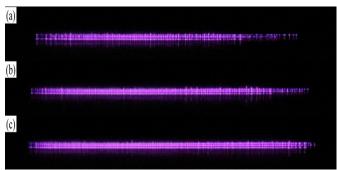
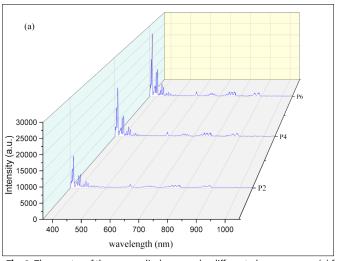


Fig. 2. Plasma discharge images at different powers (taken from right view): (a) 2 kW, (b) 4 kW, (c) 6 kW.

Fig. 3 illustrates the discharge spectrum intensity in the range of 360-1050 nm versus discharge power (Fig. 3 (a)) and showcases characteristic peaks in the spectrum corresponding to power changes. In Fig. 3 (a), it is evident that the dominant radiation wavelength within 360-435 nm, i.e. in the ultraviolet and visible violet light region, with considerably weaker radiations in the visible and infrared range. The characteristic wavelengths with the strong intensities include 379.32, 374.73, 404.70, 398.71, 425.77, 433.14 (in descending order of intensity), etc. Previous studies on emission spectra (17, 18), reveal that the peaks within the 360-500 nm range mainly correspond to the second positive system of nitrogen (N2, C-B). Wavelengths ranging from 500-1050 nm predominantly originated from the first positive system of nitrogen (N2, B-A), with a few resulting from oxygen excitation (for example, 776.96, 844.38 nm). Notably, regardless of the discharge powers, the maximum discharge peak consistently appears at the ultraviolet radiation of 379.32 nm. In Fig. 3 (b), characteristic peaks are shown relative to discharge power. It can be observed that at a given power, spectrum intensities (>450 nm) represent only 6%-8% of the intensity at 379.32 nm. With an increase in discharge power from 2 to 6 kW, the intensities of various wavelengths proportionally increase. This suggests that the alteration in discharge power does not change the intensity hierarchy of these peaks. This finding contrasts with a study on corona discharge (19), where the spectrum exhibited distinct characteristics in the infrared, visible and ultraviolet regions. In that study, as the discharge voltage increases, the ultraviolet and infrared regions displayed different patterns of changes.

To effectively treat soybean seeds, it is crucial to understand the non-uniformity of corona discharge plasma, as shown in Fig. 4. The discharge electrodes, each



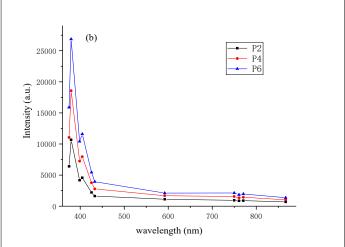
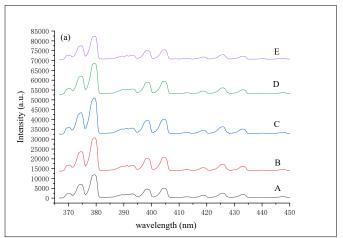


Fig. 3. The spectra of the corona discharge under different plasma powers: (a) full spectrum diagram in 360–1050 nm, (b) the intensities of some strong characteristic peaks change as discharge power.



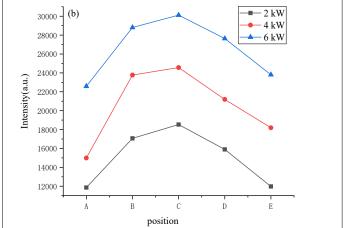


Fig. 4. (a) Discharge spectrum (2 kW) and (b) the intensity distribution of different positions A-E under different discharge powers (379.32 nm).

measuring 60 mm, are evenly divided into 4 sections labelled A, B, C, D and E from left to right (as shown in Fig. 1 (c)). In Fig. 4 (a), the spectrum curves measured at these five positions under 2 kW are presented. It is evident that the discharge intensity at each wavelength is strongest at the center of the electrode (point C), and gradually decreases symmetrically towards both sides. Fig. 4 (b) illustrates the relationship between the spectral intensity and spatial position, using the strongest peak of 379.32 nm as an example, demonstrating an approximately centrally symmetric distribution. The changes in intensity of 379.32 nm at different points under various discharge powers are shown in Table 2. For example, under 2 kW, the intensity at the center position (point C) is the highest, while at points B (D), the average intensity is approximately 88.9% of point C, and at point A (E), it's about 64.3% of point C. With an increase in discharge power to 6 kW, the intensity ratio I_{b,d}/I_c rises to 93.7%. Consequently, in practical seed processing, to ensure that

Table 2. The spectrum intensity changes at different positions (379.32 nm).

| Cuana mama | Intensity | Intensity | Intensity |
|------------|---|-----------------------|-----------------------|
| Group name | С | B, D | A, E |
| P2 | I _{c2} | 88.9% I _{c2} | 64.3% I _{c2} |
| P4 | I_{c4} (=1.33 I_{c2}) | 91.5% I _{c4} | 67.5% I _{c4} |
| P6 | I _{c6} (=1.625 I _{c2}) | $93.7\%I_{c6}$ | 57.4% I _{c6} |

seeds receive over 90% of plasma radiation, they should be distributed within the BD range.

Fig. 5 displays photos of soybean seeds treated with various plasma powers. Observing the images, it is apparent that, under the given discharge powers, the surface of the soybean seeds remained intact, with no signs of peeling, cracking, or blackening due to plasma treatment. Visually and graphically, it is challenging to distinguish between plasma-treated and untreated seeds. Subsequent experiments will assess the effects of different plasma power through germination and growth experiment.

Water absorption of soybean seeds

Fig. 6 depicts the average water absorption ratio of soybean seeds treated with various discharge powers. The water absorption ratio represents the amount of water absorbed by the seeds relative to their own weight. In Fig. 6, it is evident that, within the same timeframe, plasma treated seeds exhibited significantly higher water absorption ratio compared to untreated seeds, with absorption increasing alongside discharge power. For example, within the first 2 hr, the water absorption for group CK was 18.2%, while that for group P2, P4 and P6 was 28.0%, 35.5% and 42.9%, respectively. Similar findings have been reported in barley research (20), where LTP treatment significantly improved barley seeds, attributed to the erosion of seed coat surface observed under scanning electron



Fig. 5. Four groups of soybean seeds after treated with different discharge powers.

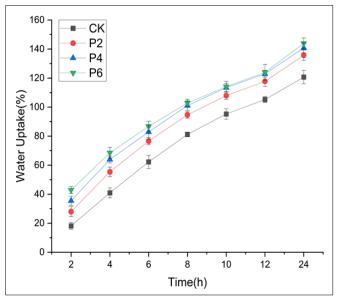


Fig. 6. Water absorption of soybean seeds treated by different plasma powers.

microscopy. Many authors have explained the enhanced water absorption of soybean seeds due to plasma treatment. Modifications in seed coat, including changes in morphology and chemistry, affect seed wettability, which is closely related to water permeability. Plasma treatment erodes the seed epidermis due to bombardment by reactive species, leading to increased seed volume ratio and enhanced wettability (19). Additionally, the interaction between plasma and seed coat components leads to degradation of cutin and wax layers, reducing the hydrophobicity and increasing water permeability (21). Observation of water droplet deposited on soybean seeds revealed a significantly decrease in apparent contact angle after plasma treatment, indicating improved water absorption (6). During plasma treatment, active functional groups are enriched and grafted onto the seed surface, significantly improving wettability and consequently affecting germination (16). Furthermore, it is noticeable that as plasma processing power increases, the increase in seed water absorption becomes progressively smaller, as shown in Table 3. For example, when plasma power increased from 0 to 2 kW, seed water absorption increased by 12.5%. With a continuous increase in plasma power from 2 to 4 and then to 6 kW, the total water absorption of seeds increased by 3.7% and 2.2%, respectively. This trend is mainly due to the gradual saturation of water absorption during the germination process.

Table 3. Comparison of total water absorption of different groups of seeds.

| Group | Total water absorption (%) | Increased from the previous group (%) |
|-------|----------------------------|---------------------------------------|
| СК | 120.66 | / |
| P2 | 135.71 | 12.5 |
| P4 | 140.75 | 3.7 |
| P6 | 143.88 | 2.2 |

Growth of soybean seedlings

The germination and growth of the seeds were monitored daily. In Fig. 7, growth images of the seedlings on the 1st, 3rd, 5th, and 7th day after seed soaking are presented. Clearly, under the same growing environment, plasma treatment significantly influences the germination and growth of soybean seeds, a phenomenon closely linked to the plasma treatment power. Notably, seedling growth shows a marked improvement with 2 kW treatment. The most optimal plasma promotion effect is observed at 4 kW, characterized by the maximum growth rate and luxuriance of the seedlings. However, at 6 kW, plasma induced growth inhibition becomes evident. This indicates that the impact of plasma treatment is dose-dependent, consistent with previous findings (22, 23). At an appropriate treatment dose, the best promotion effect is achieved, leading to enhanced emergence rate and significantly increased yield. Beyond a certain dose, plasma exerts an inhibiting effect. According to previous studies (24), suitable plasma treatment enhances water absorption by eroding the seed epidermis. However, excessive water absorption due to overdosing reduces germination rates or asphyxiates embryos, hindering germination. Moreover, high dose of plasma results in ultraviolet radiation, which might contribute to the low germination rate in seeds treated with high dose (7). Therefore, plasma seed treatment exhibits a bipolar effect. Following treatment with different plasma powers, the seedlings display varying growth states, closely tied to the seed's different vigor. Hence, our next step will involve a detailed study of the vigor indicators of these seeds.

Soybean seed vigor indicators

Fig. 8 illustrates the germination ratio of soybean seedlings during the first five days. It is evident that from the first day that distinct treatment effects are observed. On the first day, group P4 exhibited the most significant promotion (5.67 times that of group CK), and group P2 fol-

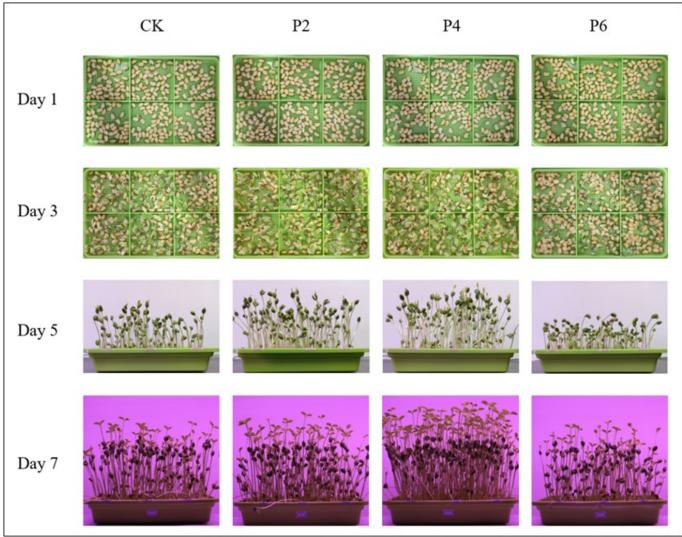
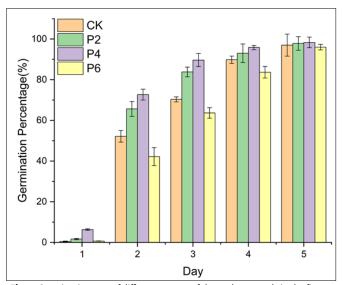


Fig. 7. Comparison of different groups of soybean seedlings.

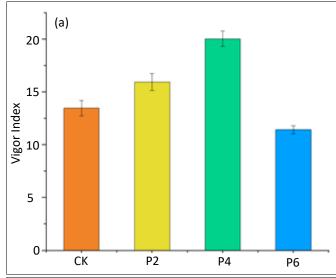


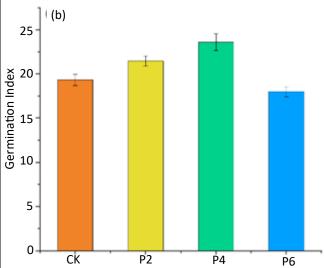
 ${\bf Fig.~8}.$ Germination rate of different groups of the soybean seeds in the first five days.

lowed as the second (1.56 times that of group CK). As time progressed, the effects of different treatment powers on the germination rate (whether promotion or inhibition) remained consistent each day, with group P4 consistently performing the best, followed by P2, CK, and P6. Another notable observation is that as time passed, the differences in germination rates resulting from different plasma powers gradually diminished. For instance, from the 1st to

the 2nd and 5th day, the germination rate of group P4 was 5.6, 1.35, and 1.04 times that of group CK, respectively. Similar trends were observed between any two comparable groups. By the 5th day, the germination rates of all four groups were closely aligned. This suggests that plasma treatment had a more pronounced effect in the very early stages of germination, possibly contributing to the varying growth statuses observed during the seedling stage.

In Fig. 9, a comparison of the average vigor index, average germination index, and average fresh weight of seedlings on the 8th day of germination is presented. These three indexes show similar trends with plasma power: group P4 exhibited the best effects, followed by P2, CK, and P6. When combined with Fig. 7, it is evident that these three indicators follow the same pattern as plasma power. Previous studies have indicated that lowtemperature plasma treatment not only impacts shortterm germination rates but also influences long-term stem and root growth (22). Consequently, these indicators are closely related to seed vigor, representing a comprehensive measure of seed quality, encompassing germination rates, emergence rates, seedling growth potential, and production potential. The bipolar relationship between seed vigor and plasma treatment power explains the differences observed in germination, growth, and fresh weight in Fig. 7-9.





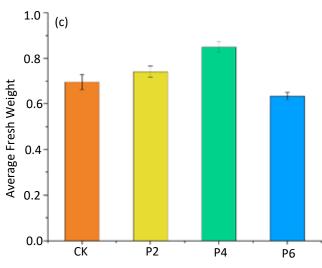


Fig. 9. Comparison on the four groups: (a) average vigor index, (b) average germination index, (c) average fresh weight of seedlings.

Conclusion

This study conducted experiments on soybean seed treatment using corona plasma and examined the influence of various plasma power levels on soybean seed vigor. Analysis of the discharge spectrum revealed characteristic wavelengths predominantly in the ultraviolet region. Importantly, the increase in discharge power did not alter

the distribution pattern of spectral intensity across wavelengths. The spatial distribution of the discharge indicated that approximately 90% of the spectral intensity concentrated within the central half of the discharge region. To assess the effects of different plasma powers, germination index, seedling growth, and fresh weight were utilized as characterization parameters. The findings demonstrated that appropriate plasma treatment could enhance seed viability and facilitate seedling growth. For instance, after being treated with a 4 kW corona plasma for 4.5 s, soybean seeds exhibited a 4.6-fold increase in germination rate on the first day, and the seedlings' fresh weight surged by almost 30% after 5 days of sowing. Therefore, plasma treatment proved effective in promoting both germination and growth of soybeans, ultimately improving soybean yield. However, excessive treatment resulted in inhibitory effects. These results underscore the practicality and effectiveness of corona plasma treatment for soybean seeds.

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Authors contributions

Conceptualization, LL and FH; methodology, LL and FH; software, XL, RL and BL; validation, LL, MW and WZ; formal analysis, XT and DZ; investigation, WC and DZ; resources, FH; data curation, LL and GS; writing-original draft preparation, ER, FH; writing-review and editing, FH and RL; visualization, JG and MW; supervision, FH; project administration, FH; funding acquisition, FH. All authors have read and agreed to the published version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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