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Improvement of Wheat Seed Vitality by Dielectric Barrier Discharge Plasma Treatment

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Influences of discharge voltage on wheat seed vitality were investigated in a dielectric barrier discharge (DBD) plasma system at atmospheric pressure and temperature. Six different treatments were designed, and their discharge voltages were 0.0, 9.0, 11.0, 13.0, 15.0, and 17.0 kV, respectively. Fifty seeds were exposed to the DBD plasma atmosphere with an air flow rate of 1.5 L min^{-1} for 4 min in each treatment, and then the DBD plasma-treated seeds were prepared for germination in several Petri dishes. Each treatment was repeated three times. Germination indexes, growth indexes, surface topography, water uptake, permeability, and α-amylase activity were measured. DBD plasma treatment at appropriate energy levels had positive effects on wheat seed germination and seedling growth. The germination potential, germination index, and vigor index significantly increased by 31.4%, 13.9%, and 54.6% after DBD treatment at 11.0 kV, respectively, in comparison to the control. Shoot length, root length, dry weight, and fresh weight also significantly increased after the DBD plasma treatment. The seed coat was softened and cracks were observed, systematization of the protein was strengthened, and amount of free starch grain increased after the DBD plasma treatment. Water uptake, relative electroconductivity, soluble protein, and α-amylase activity of the wheat seed were also significantly improved after the DBD plasma treatment. Roles of active species and ultraviolet radiation generated in the DBD plasma process in wheat seed germination and seedling growth are proposed. Bioelectromagnetics. 39:120–131, 2018. © 2017 Wiley Periodicals, Inc

Keywords: air plasma; dielectric barrier discharge; seed germination; seedling growth; wheat seed

INTRODUCTION

Seeds, as living organisms, are the most basic and important means in crop production. Good quality seeds are characterized by high seed vigor, which ensure rapid germination, robust seedlings, and uniform growth in the field [Ashrafi and Razmjoo, 2015; Miano et al., 2015]. High germination rate, high emergence rate, a full stand, and robust seedlings are essential to yield [Adetimirin, 2008]. Therefore, it is necessary to choose good quality seeds to obtain high yield. Generally, there has been approximately a 5–40% decrease in yield for seeds with low vigor compared with seeds with high vigor [Adetimirin, 2008; Mondo et al., 2013; Tiwari et al., 2015]. Hence, there is important practical significance to enhance seed vigor and obtain high-quality seeds in agricultural production.

Several seed improvement methods have been employed to enhance seed vigor, including chemical

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treatments (chemicals, fungicides, and hormones) [Zehra et al., 2013; Zhao and Jiang, 2014] and physical treatments (ultrasonic scratching, electric field treatment, magnetic treatment, ion beam scratching, etc.) [Goussous et al., 2010; Xu et al., 2012; Shi et al., 2014; Yao and Shen, 2015]. Chemical methods are usually labor-intensive and expensive due to large amounts of chemical consumption; even worse, residual chemicals on the seed coat surface could contribute to soil pollution. Compared to chemical treatments, more attention has recently been paid to physical treatments. As physical methods, ultrasonic scratching and ion beam scratching could improve seed vigor, and electric and/or magnetic treatments have also been confirmed to enhance seed vigor by influencing biochemical processes such as protein and enzyme activity [Moon and Chung, 2000]. However, these physical methods have limitations; for example, strong ultrasonic oscillation or ion beam collision might injure seed cells, thus increasing the number of destroyed seeds, and physical methods could lead to a higher possibility of a non-uniform treatment [Goussous et al., 2010; Xu et al., 2012; Shi et al., 2014; Yao and Shen, 2015].

Cold plasma treatment has been a fast, economical, and green method to enhance seed performance [Denes et al., 1999; Yin et al., 2005; Dhayal et al., 2006; Sera et al., 2010; Li et al., 2014]. Previous studies demonstrated that cold plasma treatment could improve seed germination and fast growth of several plants such as *Carthamus tinctorium* L. [Dhayal et al., 2006], wheat [Sera et al., 2010], tomato [Yin et al., 2005], soybean [Li et al., 2014], poppy seed [Sera et al., 2013], and cannabis [Sera et al., 2016], and it could significantly increase crop yields [Yin et al., 2005]. There was no destruction of seeds for the cold plasma treatment because the active species only penetrated approximately 10 nm deep, thus the seed surfaces could only be functionalized with plasma [Denes et al., 1999; Dhayal et al., 2006]. Additionally, no environmental pollutants remained after seed treatment by the cold plasma because it did not require chemicals. The improvement of seeds has depended on the seed features of specific plants [Denes et al., 1999; Yin et al., 2005; Dhayal et al., 2006; Sera et al., 2010; Li et al., 2014], plasma generation techniques (such as microwave plasma, radiofrequency discharge plasma, high-frequency discharge plasma, and magnetized arc discharge plasma) [Yin et al., 2005; Dhayal et al., 2006; Li et al., 2014; Sera et al., 2016], and gas sources of the plasma (such as argon, helium, CF_4, aniline, hydrazine, and nitrogen) [Volin et al., 2000; Dhayal et al., 2006; Li et al., 2014; Ji et al., 2015]. Dielectric barrier discharge (DBD) plasma, one type of cold plasma technique, could be easily triggered under atmospheric pressure by applying high voltage to electrodes, one of which was at least covered with dielectric material [Ji et al., 2015]. DBD plasma could generate ultraviolet radiation (UV), strong electric field, high energy electrons, and various active radicals, all of which have been recently used in agricultural applications because it has caused little damage to materials and does not need a rare gas source or a complicated vacuum system [Kitazaki et al., 2014]. Tong et al. [2014] reported that seed germination and early growth of *Andrographis paniculata* was enhanced by a DBD plasma treatment with air as the gas source. However, little research has been conducted on wheat seed improvement by atmospheric pressure DBD plasma treatment.

Wheat has been one of the most important grain crops, and has been widely cultivated worldwide as a staple food. It is also the second largest staple food in China, and its demand has unceasingly increased with the decrease of arable land area and the increase of population. Enhancing wheat seed vigor and obtaining high-quality wheat seed has become an urgent problem in China [Tong et al., 2014]. Therefore, the aim of this study was to obtain information about the influence of DBD plasma treatment on wheat seed germination and early seedling growth. We focused on investigating the effects of DBD plasma treatment on seed germination, early seedling growth, surface changes of the seed coat, permeability, the content of soluble protein, and α-amylase activity in young seedlings. The mechanisms of promoting wheat seed germination and seedling growth via DBD plasma treatment were also explored. It was expected to provide an efficient and viable approach for wheat seed improvement using cold plasma.

MATERIALS AND METHODS

Plant Material

Wheat seed (Xiaoyan 22, Yangling, Shaanxi, China) was obtained from the Seed Research Institute of Northwest A&F University. Size uniform and plump seeds with no injuries in the embryo were carefully chosen, disinfected for 10 min by 0.2% $HgCl_2$ solutions, and then washed with autoclaved distilled water three times. Subsequently, the water on the surface of the wheat seeds was absorbed using suction filter paper, and then they were dried in an oven at 30 °C for 2 days; and finally, the dried seeds were stored at 0–5 °C in a refrigerator for use.

DBD Plasma Generation and Seed Treatment

The schematic diagram of the experimental apparatus for the seed treatment is illustrated in Figure 1; the apparatus consisted of an alternating current high-voltage power supply and a reactor vessel, which was similar to our previous research [Meng et al., 2017]. The power supply was made by the Institute of Electrostatics and Special Power, Dalian University of Technology, China, the discharge voltage was adjustable from 0 to 50 kV, and the frequency of the power supply was 50 Hz. The DBD experimental chamber for the wheat seed treatment was made of a Plexiglas cylinder (100 mm inner diameter and 8 mm height). The high voltage electrode was a stainless steel plate (120 mm diameter and 2 mm thickness), which was covered by a 1.5 mm-thick quartz glass plate as a dielectric barrier (180 mm diameter). The ground electrode was a wire netting (40 mesh), which was embedded into a Plexiglas cylinder (100 mm inner diameter). The gap space between the high voltage electrode and ground electrode was 8 mm.

Six different treatments were designed in this research for seed treatment, and their discharge voltages were 0.0, 9.0, 11.0, 13.0, 15.0, and 17.0 kV, respectively; correspondingly, these treatments were marked as CK, T1, T2, T3, T4, and T5 treatments, respectively. In each treatment, 50 wheat seeds were unilaminar spread flat out on the ground electrode of the DBD plasma chamber, dry air with a gas flow rate of 1.5 L min^{-1} was injected into the DBD plasma chamber and vented through the ground electrode, and then the DBD plasma was triggered via putting a certain discharge voltage across the electrodes. For each treatment, the treatment time was 4.0 min, which has already been optimized in our previous research [Meng et al., 2017]. Each treatment was replicated three times.

Seed Germination Design

After the DBD plasma treatment, the 50 wheat seeds were soaked in deionized water for 5 h at 20 °C for accelerating germination, disinfected for 2 min by 70% alcohol, and then followed by a rinse with autoclaved distilled water and prepared for germination.

The seed germination test was conducted in each autoclaved Petri dish (diameter 9 cm); two pieces of autoclaved filter paper wetted by 10 mL autoclaved distilled water were used as a germinating bed, the 50 wheat seeds with groin downward were sown orderly in a Petri dish, then the total weight of the petri dish containing these seeds was weighed. Afterwards, the Petri dish with lid was moved to a germination incubator and the germination conditions were set at 20 °C with a 12 h light/12 h dark photoperiod at a photo flux density of 120 μmol m^{-2} s^{-1} and 70% air humidity. There were six treatments, each replicated three times; therefore, there were 18 Petri dishes in this research. The germination period was 4 days. During the whole germination period, the total weight of the Petri dish was weighed every day, and then autoclaved distilled water was added to each Petri dish to make up the water loss. On the third day of germination, the lid of the Petri dish was taken away to avoid its block for seedling growth. A radicle protrusion of 1 mm was recorded as the criterion for germination. The germination potential was calculated at the first day of plant (24 h after plant), and germination rate was calculated every 24 h of plant. Fifteen seedlings from each Petri dish were randomly taken for total root length, shoot length, fresh weight,

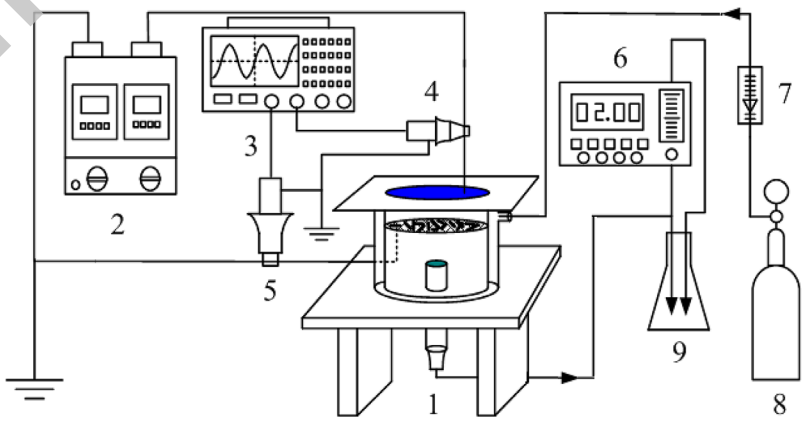

Fig. 1. Schematic diagram of discharge plasma reaction system (1. reactor; 2. power source; 3. oscilloscope; 4. high voltage probe; 5. current probe; 6. ozone tester; 7. flow meter; 8. gas source; 9. potassium iodide solutions).

dry weight, soluble protein, and α-amylase activity measurement on the fourth day.

Analytical Methods

The percentage of germination was calculated using the following equations [Tong et al., 2014]:

$$\text{Germination potential } (\%) = \frac{\text{number of seeds germinated in 1 d}}{\text{total number of seeds}} \times 100\% \quad (1)$$

$$\text{Germination rate } (\%) = \frac{\text{number of seeds germinated in 4 d}}{\text{total number of seeds}} \times 100\% \quad (2)$$

$$\text{Germination index} = \sum (G_t/D_t) \quad (3)$$

$$\text{Vigor index} = \text{Rootlength (cm)} \times \text{Germination index} \quad (4)$$

where G_t represents number of germinated seeds on t day, and D_t represents germination days.

After 15 seedlings from each Petri dish were randomly sampled, their surface water was absorbed using filter paper, and then the total root length and total shoot length of these seedlings were measured using a vernier caliper. For the root length measurement, all the roots of one seedling were measured. After the surface water of the seedlings was absorbed by filter paper, their fresh weight was immediately measured. Then the seedlings were dried overnight in an oven at 80 °C, and their dry weights were measured.

Determination of seed relative electroconductivity and water uptake was based on the method given by Yang and Shen [2011]. Both controlled and DBD plasma-treated seeds were soaked in deionized water for 48 h, and the electroconductivity of the leachate solutions was tested and recorded as S_1. After that, the leachate solutions together with the seeds were boiled for 20 min and then cooled to test the electroconductivity, which was recorded as S_2. The electroconductivity of the deionized water was tested and recorded as S_0, and the relative electroconductivity was calculated as follows:

$$\text{Relative electroconductivity} = \frac{S_1 - S_0}{S_2 - S_0} \times 100\% \quad (5)$$

For the water uptake measurement, the dry seeds were first weighed and recorded as m_0. Then they were soaked in deionized water for 48 h and weighed and recorded as m_1 after absorbing the surface water on the seeds. The water uptake was calculated using the following equation:

$$\text{Water uptake } (\%) = \frac{m_1 - m_0}{m_0} \times 100\% \quad (6)$$

SEM S-4800 (Hitachi, Tokyo, Japan) was applied to characterize the morphology and structure of the control and DBD plasma-treated seeds. For the outside surface appearance analysis, dry seeds were directly analyzed by SEM. For the cross section properties analysis, the seeds were first sliced by a single-edge blade and an Au film deposited by ion sputtering equipment, and then analyzed by SEM.

To measure the soluble protein, 15 seedlings at the fourth day of plant were randomly sampled, and the total fresh weight of their coleoptiles was measured. The frozen coleoptiles were ground in liquid nitrogen, and the powder was suspended in 5 mL 0.05 mol L^{-1} sodium phosphate buffer (pH 7.8) containing 1 mmol L^{-1} EDTA and protease inhibitor. Then the mixture was centrifuged at 12,000 r/min for 15 min at 4 °C, and finally the supernatant was used for the soluble protein measurement. One mL supernatant was sampled, 5 mL coomassie brilliant blue solutions were added, and then absorbance was recorded at 595 nm [Bradford, 1976]. The concentration of the soluble protein was expressed as mg g^{-1} fresh weight of the coleoptiles.

To measure α-amylase activity, 15 frozen seedlings at the fourth day of plant were ground in liquid nitrogen, and the powder was suspended in 5 mL 0.05 mol L^{-1} acetate buffer. Then the mixture was centrifuged at 12,000 r/min for 15 min at 4 °C, and finally the supernatant was taken for measurement. α-Amylase preparations were conducted following the procedure for total amylase with the exception that heating was at 70 °C for 15 min. The substrate for amylase was 1% solution of soluble starch in 0.1 mol L^{-1} acetate buffer. After incubation at 25 °C for 1 h, 2 mL 3,5-dinitrosalicylic acid was added, and the mixtures were put in a boiling water bath for 5 min then cooled to room temperature. The absorbance value at 540 nm was determined using a spectrophotometer. A standard curve was plotted using maltose as a standard [He et al., 2010]. The α-amylase activity was calculated as follows:

$$\alpha\text{-amylase activity}(\text{mg g}^{-1}\text{min}^{-1}) = \frac{M \cdot V_t}{W \cdot t \cdot V_s} \quad (7)$$

where M is maltose content, V_t is total volume of the enzyme extract, V_s is volume of the extract used for the reaction, W is fresh weight of the seedlings, and t is the reaction time.

All treatments contained three replicates. The data in this research were presented as the mean value ± standard error of mean of three repeated experiments. SPSS statistical software (Version 16.0; Bizinsight Limited Company, Beijing, China) and one-way analysis of variance (ANOVA) were used to confirm variability of the data and validity of the results. Differences among treatments were compared using Duncan's multiple range tests at 0.05 probability level, as reported by Li et al. [2014]. In the figures, the spread of the values are shown as error bars representing standard errors of the means.

RESULTS

Discharge Characteristics During Seed Treatment by DBD Plasma

UV light was observed during the wheat seed treatment by the DBD plasma, and the wheat seeds were covered by UV irradiation as shown in Figure 2a. Optical emission spectroscopy was used to detect active species during the wheat seed treatment by the DBD plasma; the emission spectrum of DBD in the range of 300–800 nm is shown in Figure 2b. N-containing species and O radicals were detected in the present DBD plasma system.

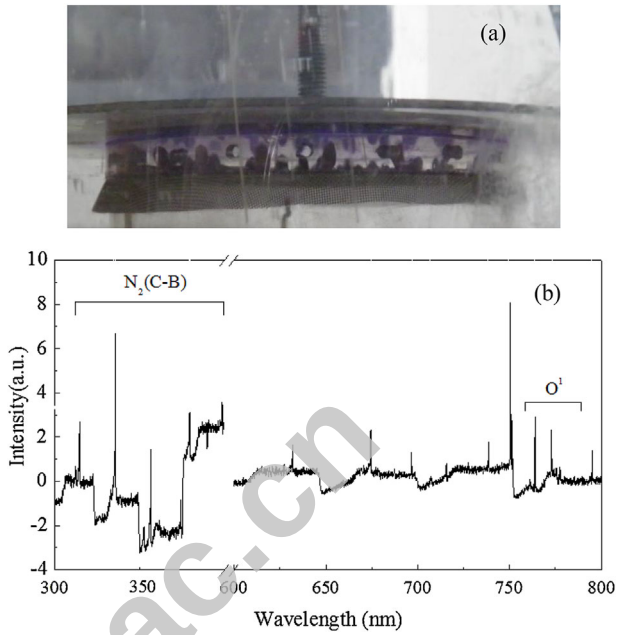

Fig. 2. Typical discharge photograph and emission spectrum of DBD in the range of 300–800 nm under seed treatment conditions (a. discharge photograph; b. emission spectrum).

Wheat Seed Germination

The results of wheat seed germination after DBD plasma treatment are shown in Table 1, and the analysis of variance of germination parameters is shown in Table 2. Specifically, the mean germination potential was 77.2%, 82.1%, 77.5%, 74.9%, and 72.0% for the T1, T2, T3, T4, and T5 treatments, respectively, and the treatments exhibited significant differences compared with the CK (the mean germination potential was 62.5%). There was no significant difference among the T1, T2, T3, and T4 treatments, but the difference among the T2 and T5 treatments was quite significant. The highest mean germination potential was obtained in the T2 treatment.

The mean germination rate was 88.0% and 91.4% for the CK and T1 treatments, respectively, and there was no significant difference among them. The mean germination rate increased to 94.7% and 95.3% for the T2 and T3 treatments, respectively, and these treatments exhibited significant differences compared with the CK; however, there was no significant difference among the T2 and T3 treatments. The mean germination rate was 90.5% and 90.7% for the T4 and T5 treatments, respectively, and there was no significant difference among the CK, T4, and T5 treatments; however, there was a significant difference among the T3 and T5 treatments.

The mean germination index increased to 40.6, 41.8, 41.3, and 40.2 for the T1, T2, T3, and T4 treatments, respectively, and these treatments presented significant differences compared with the CK (the mean germination index was 36.7); however, there was no significant difference among the T1, T2, T3, and T4 treatments. The mean germination index was 39.8 for the T5 treatment, which did not exhibit any significant difference compared with other treatments. The highest mean germination index reached 41.8 for the T2 treatment ($P \leq 0.026$).

The mean vigor index increased to 546.4, 595.4, 493.3, and 500.5 for the T1, T2, T3, and T4 treatments, respectively, and these treatments presented significant differences compared with the CK (the mean vigor index was 385.2); that is, the vigor index increased by 41.8% ($P \leq 0.003$), 54.6% ($P \leq 0.001$), 28.1% ($P \leq 0.025$), and 29.9% ($P \leq 0.004$) after the T1, T2, T3, and T4 treatments, respectively. The highest mean vigor index was obtained in the T2 treatment, which presented a significant difference compared with the T3 or T4 treatment. The mean vigor index was 440.0 for the T5 treatment, and there was no significant difference compared with the CK; however, there was a significant difference among the T5 and T2 treatments.

TABLE 1. Effect of Discharge Voltage on Germination Characteristics

Treatment	Germination potential (%)	Germination rate (%)	Germination index	Vigor index
CK	62.5 ± 6.1c	88.0 ± 2.0c	36.7 ± 1.0b	385.2 ± 15.4d
T1	77.2 ± 4.2ab	91.4 ± 2.5abc	40.6 ± 1.6a	546.4 ± 43.5ab
T2	82.1 ± 5.1a	94.7 ± 2.9ab	41.8 ± 2.9a	595.4 ± 36.6a
T3	77.5 ± 4.3ab	95.3 ± 3.2a	41.3 ± 1.8a	493.3 ± 64.8bc
T4	74.9 ± 2.5ab	90.5 ± 0.9bc	40.2 ± 0.9a	500.5 ± 21.0b
T5	72.0 ± 3.5b	90.7 ± 1.2bc	39.8 ± 1.7ab	440.0 ± 27.6cd

Different lowercase letters mean significant difference among different treatments at $P \leq 0.05$ level.

In addition, for germination potential, germination rate, and vigor index, there was a significant difference between groups, as shown in Table 2.

Seedling Growth at Different Discharge Voltages

The effects of the DBD plasma treatment on fresh weight, dry weight, shoot length, and root length of the wheat seedlings are shown in Table 3. The mean fresh weights of the seedlings for the T1, T2, T3, and T4 treatments were 2.252, 2.279, 2.2045, and 2.195 g, respectively, which was 16.2% ($P \leq 0.01$), 17.6% ($P \leq 0.01$), 13.8% ($P \leq 0.044$), and 13.3% ($P \leq 0.034$) higher than that of the CK, respectively; there was no significant difference among the T1, T2, T3, and T4 treatments. The mean fresh weight was 2.039 g for the T5 treatment, and there was no significant difference compared with the CK; however, there was a significant difference among the T5 and T2 treatments. The highest mean fresh weight was obtained in the T2 treatment.

TABLE 2. Analysis of Variance of Germination and Seedling Growth Parameters

Index	Source of variance	df	Mean square
Germination potential	Between groups	5	135.21*
	Within groups	12	15.13
Germination rate	Between groups	5	23.25*
	Within groups	12	5.05
Germination index	Between groups	5	9.54
	Within groups	12	3.13
Vigor index	Between groups	5	16,684.9*
	Within groups	12	976.6
Fresh weight	Between groups	5	0.054*
	Within groups	12	0.009
Dry weight	Between groups	5	0.001*
	Within groups	12	0.000
Root length	Between groups	5	123,514*
	Within groups	12	2,408
Shoot length	Between groups	5	1,091.9*
	Within groups	12	126.0
Water uptake	Between groups	5	91.96*
	Within groups	12	16.02
Relative electroconductivity	Between groups	5	48.20*
	Within groups	12	8.28
α-Amylase activity	Between groups	5	0.11*
	Within groups	12	0.02
Soluble protein	Between groups	5	9.34*
	Within groups	12	1.76

The significant level was set as $P \leq 0.05$.
*Represents significant.

The mean dry weight was 0.678 and 0.682 g for the CK and T1 treatments, respectively, and there was no significant difference among them. The mean dry weight increased to 0.703, 0.722, and 0.713 g for the T2, T3, and T4 treatments, respectively, and these treatments exhibited significant differences compared with the CK. There also presented significant difference among the T2 and T3 treatments, thus the highest mean dry weight was obtained in the T3 treatment. In addition, the mean dry weight was 0.690 g for the T5 treatment, and there was no significant difference among the T5 and CK treatments, whereas the difference among the T5 and T3 treatments was significant.

The mean shoot length increased to 510.3, 523.7, 490.9, and 499.8 mm for the T1, T2, T3, and T4 treatments, respectively, and these treatments presented significant differences compared with the CK (the mean shoot length was 468.8 mm). The highest mean shoot length was obtained in the T2 treatment, which presented a significant difference compared with the T3 or T4 treatment, although the difference among the T2 and T1 treatments was not significant. The mean shoot length was 487.5 mm for the T5 treatment, and there was no significant difference compared with the CK; however, there was a significant difference among the T5 and T2 treatments.

The mean root length was 1,573.7, 1,999.7, 2,094.0, 1,759.3, and 1,863.3 mm for the CK, T1, T2, T3, and T4 treatments, respectively, and the difference among these treatments was significant, and the highest mean root length was obtained in the T2 treatment. The mean root length was 1640.3 mm for the T5 treatment, and there was no significant

TABLE 3. Effect of Discharge Voltage on Seedling Growth of Wheat Seed

Treatment	Fresh weight (g)	Dry weight (g)	Shoot length (mm)	Root length (mm)
CK	1.938 ± 0.057c	0.678 ± 0.006c	468.8 ± 8.3d	1,573.7 ± 79.8e
T1	2.252 ± 0.097a	0.682 ± 0.006c	510.3 ± 15.7ab	1,999.7 ± 20.8b
T2	2.279 ± 0.035a	0.703 ± 0.011b	523.7 ± 5.8a	2,094 ± 25.0a
T3	2.205 ± 0.149ab	0.722 ± 0.009a	490.9 ± 5.1bc	1,759.3 ± 46.4d
T4	2.195 ± 0.129ab	0.713 ± 0.012ab	499.8 ± 12.8bc	1,863.3 ± 40.4c
T5	2.039 ± 0.062bc	0.690 ± 0.013c	487.5 ± 14.9 cd	1,640.3 ± 92.5e

Different lowercase letters mean significant difference among different treatments at $P \leq 0.05$ level.

difference compared with the CK; however, there existed a significant difference among the T5 and T2 treatments.

In addition, the analysis of variance of seedling growth parameters is also shown in Table 2. For the dry weight, fresh weight, shoot length, and root length, there was a significant difference between groups.

Scanning Electron Microscope (SEM) Analysis of Seeds

The surface properties of the wheat seeds are shown in Figure 3. The seed coat presented a rectangular type of sub-domain with a clear boundary layer before the DBD plasma treatment (Fig. 3a and c), whereas the rectangular type of the sub-domain was turned into a softer structure, and its boundary layer was also difficult to identify after the DBD plasma treatment (Fig. 3b and d). In addition, cracks were observed on the seed coat after the DBD plasma treatment (Fig. 3d arrowed).

The cross section properties of the wheat seeds are also shown in Figure 3. The protein presented a complex reticular formation in the endosperm of the seed, and some starch grain was encapsulated in the protein's reticular formation while other starch grain was dissociative (Fig. 3e and g). The systematization of the protein was strengthened after the DBD plasma treatment, where lots of the starch grain had escaped from the protein's reticular formation, which increased the amount of free starch grain. In addition, it was relatively easy to distinguish the boundary of protein and starch grain after the treatment (Fig. 3f and h).

Water Uptake and Relative Electroconductivity

The effects of the DBD plasma treatment on water uptake and relative electroconductivity of the wheat seeds are shown in Table 4. The mean water uptake was 41.2% and 47.8% for the CK and T1 treatments, respectively, and there was no significant difference among them. The mean water uptake increased to 53.0%, 56.8%, and 51.3% for the T2, T3, and T4 treatments, respectively, and these treatments exhibited significant differences compared with the CK. However, there was no significant difference among these treatments. The mean water uptake was 45.8% for the T5 treatment, and there was no significant difference among the T5 and CK treatments; however, the difference among the T5 and T3 treatments was significant, and the highest mean water uptake was obtained in the T3 treatment.

The mean relative electroconductivity was 51.6%, 54.1%, 56.5%, and 50.8% for the T1, T2, T3, and T4 treatments, respectively, and these treatments presented significant differences compared with the CK (the mean relative electroconductivity was 44.8%). The highest mean relative electroconductivity was obtained in the T3 treatment, which presented a significant difference compared with the T4 treatment, although the difference among the T1, T2, and T3 treatments was not significant. The mean relative electroconductivity was 49.5% for the T5 treatment, and there was no significant difference compared with the CK; however, there was a significant difference among the T5 and T3 treatments.

In addition, the analysis of variance of seedling growth parameters is also shown in Table 2. For the relative electroconductivity and water uptake, there was a significant difference between groups.

Soluble Protein Content and α-Amylase Activity

The influence of DBD plasma on soluble protein content and α-amylase activity of the wheat seedlings is shown in Table 4. The mean α-amylase activity was 1.10 mg g^{-1} min^{-1} and 1.35 mg g^{-1} min^{-1} for the CK and T1 treatments, respectively, and there was no significant difference among them. The mean α-amylase activity increased to 1.52, 1.66, and 1.46 mg g^{-1} min^{-1} for the T2, T3, and T4 treatments, respectively, and these treatments exhibited significant differences compared with the CK; however, there was no significant difference among them. The

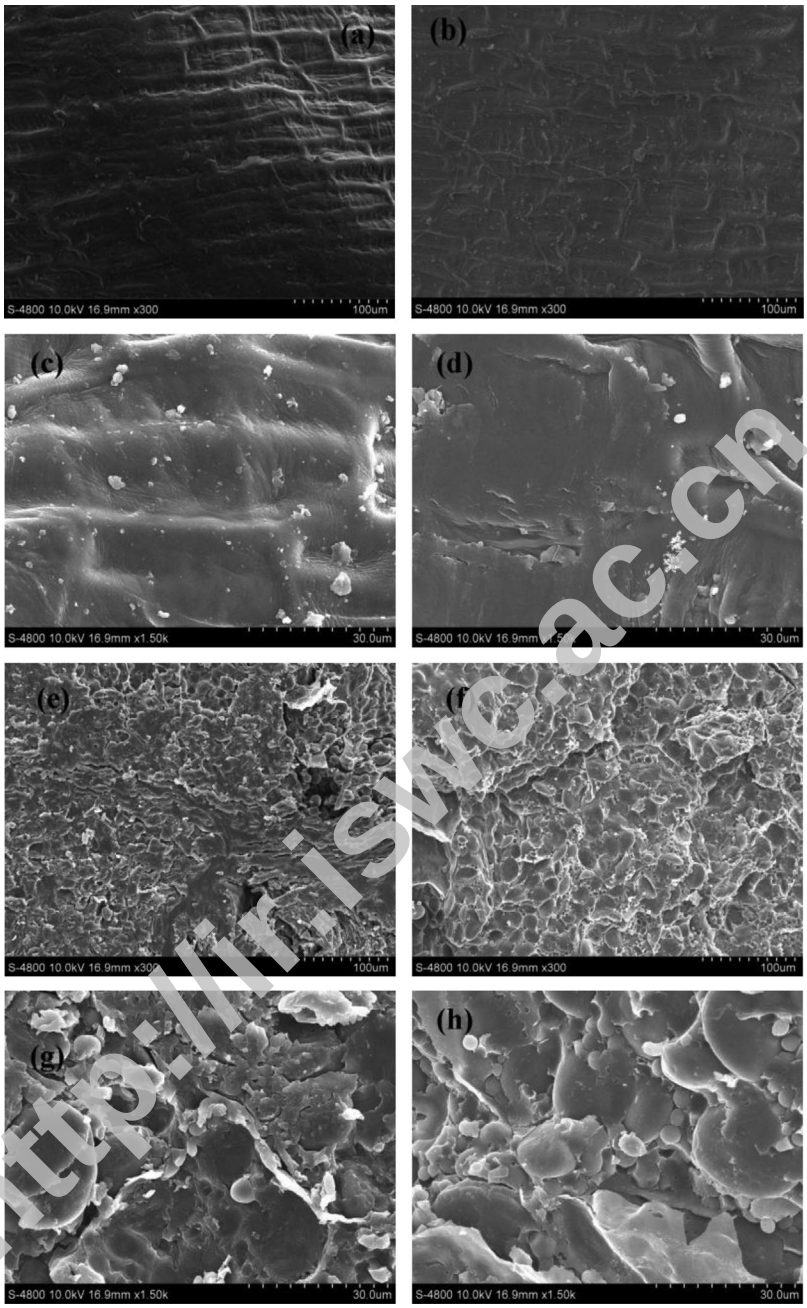

Fig. 3. SEM photographs of wheat seed (a–d: SEM photographs of wheat seed coat; a and c: before treatment; b and d: plasma treatment at 11.0 kV; arrow in d indicates cracks in seed coat; e–h: SEM photographs of cross section of wheat seed; e and g: before treatment; f and h: plasma treatment at 11.0 kV).

mean α-amylase activity was 1.34 mg g^{-1} min^{-1} for the T5 treatment, and there was no significant difference among the T5 and CK treatments; however, the difference among the T5 and T3 treatments was significant, and the highest mean α-amylase activity was obtained in the T3 treatment.

The mean soluble protein content was 30.9 and 32.1 mg g^{-1} for the CK and T1 treatments, respectively, and there was no significant difference among them. The mean soluble protein content increased to 34.1 and 35.6 mg g^{-1} for the T2 and T3 treatments, respectively, and these treatments exhibited significant differences compared with the CK; however, there was no significant difference among the T2 and T3 treatments. The mean soluble protein content was 33.3 and 31.4 mg g^{-1} for the T4 and T5

TABLE 4. Effect of Discharge Voltage on Water Uptake, Relative Electroconductivity, Soluble Protein, and α-Amylase Activity

Treatment	Water uptake (%)	Relative electroconductivity (%)	α-Amylase activity ($mg\,g^{-1}\,min^{-1}$)	Soluble protein ($mg\,g^{-1}$)
CK	41.2 ± 2.4c	44.8 ± 2.8c	1.10 ± 0.16c	30.9 ± 1.8c
T1	47.8 ± 2.6bc	51.6 ± 1.5ab	1.35 ± 0.08bc	32.1 ± 1.3bc
T2	53.0 ± 5.3ab	54.1 ± 1.5ab	1.52 ± 0.21ab	34.1 ± 0.4ab
T3	56.8 ± 3.7a	56.5 ± 3.8a	1.66 ± 0.14a	35.6 ± 1.4a
T4	51.3 ± 5.0ab	50.8 ± 3.7b	1.46 ± 0.16ab	33.3 ± 1.4abc
T5	45.8 ± 4.1bc	49.5 ± 2.9bc	1.34 ± 0.12bc	31.4 ± 1.3c

Different lowercase letters mean significant difference among different treatments at $P \leq 0.05$ level.

treatments, and there was no significant difference among the T4, T5, and CK treatments; however, the difference among the T5 and T3 treatments was significant, and the highest mean soluble protein content was obtained in the T3 treatment.

In addition, the analysis of variance of seedling growth parameters is also shown in Table 2. For the soluble protein and α-amylase activity, there was a significant difference between groups.

DISCUSSION

DBD Plasma Assessment for Enhanced Seed Germination and Growth

Lots of research has reported that cold plasma significantly increased seed germination [Yin et al., 2005; Dhayal et al., 2006; Sera et al., 2010; Li et al., 2014]. Sera et al. [2010] found that microwave plasma could increase wheat and oat germination. Stolarik et al. [2015] concluded that pea seed treated by high-frequency plasma significantly improved its germination rate. However, these results were not consistent with those reported by Volin et al. [2000], who observed that corn and bean seed germination were inhibited after a fluorocarbon plasma treatment, and attributed these phenomena to a plasma-deposition of hydrophobic materials on the seed coats. In addition, Li et al. [2014] found that cold plasma treatments with lower or higher energy levels had no significant effects on soybean germination. Tong et al. [2014] also reported that a DBD plasma treatment with higher or lower energy levels did not enhance *Andrographis paniculata* germination potential. Our research was consistent with those reported by Tong et al. [2014], thus an appropriate air DBD plasma treatment could promote wheat seed germination in laboratory conditions.

Our results were similar with those reported by Li et al. [2014], whose research on cold plasma treatments with an appropriate energy level showed improvement in soybean seedling growth. Numerous studies have suggested that cold plasma treatments promote seedling growth [Dhayal et al., 2006; Zhou et al., 2011; Sera et al., 2013]. Dhayal et al. [2006] found that the seedling growth of *Carthamus tinctorius L* was significantly increased by cold plasma treatment. Zhou et al. [2011] reported that an atmospheric pressure plasma treatment improved tomato seedling growth. Sera et al. [2013] also observed that poppy seedling growth was enhanced by a cold plasma treatment.

Mechanisms for Plasma-Induced Seed Germination and Growth

As to the problem of how the cold plasma influences plant physiological reactivity, some researchers have proposed that cold plasma-induced reactions on the seed coat could result in a deeper penetration of active species (such as reactive oxygen and N species) and UV radiation, which probably benefit physiological reactions [Grzegorzewski et al., 2010; Sera et al., 2010]. Sera et al. [2010] found that the contents of phenolic compounds in wheat and oat seeds changed after plasma treatment, and they attributed these phenomena to the penetration of plasma active species into the caryopses that subsequently affected metabolic processes. Filatova et al. [2011] reported that chemical etching effect derived from the active species of discharge plasma played an important role in stimulating the biochemical processes of seeds and influencing seed germination. Zhang and Bjorn [2009] also proposed that UV radiation was a stressor that influenced the content of phenolic compounds in organisms. UV irradiation and active radicals (Fig. 2) might also affect wheat seed germination and seedling growth in our study, as some etching effects on the wheat seed coat were observed, and morphology of the protein and starch grain were also changed (Fig. 3). The influence of the plasma treatment on seeds contained two ways; that is, there was a direct treatment on the seed coat and an effect on the cells inside the seed. The direct treatment

on the seed coat resulted in the etching effects derived from energetic ions, UV irradiation, and radicals, and the effect on the cells inside the seed involved the penetration of active radicals [Krentsel et al., 1994].

Numerous studies have suggested that there might be some association between seed germination and water uptake, and the water uptake of seeds would benefit seed germination and seedling growth [Volin et al., 2000; Stolarik et al., 2015]. Our study found that DBD plasma treatments enhanced the water uptake of wheat seed (Table 4), and these results were consistent with the findings of Filatova et al. [2011], who observed that the cold plasma treatment could improve the water uptake of some grains and legumes. Similar phenomena were observed by Bormashenko et al. [2012], who found that the water uptake of oat was improved after a radiofrequency plasma treatment. They deduced that the wetting properties of seeds were changed due to oxidation of the seed surface by the plasma treatment, which would then benefit water uptake and seed germination. Sera et al. [2010] observed that the wetting properties of oat and wheat seeds were enhanced after microwave plasma treatment, as well as their germination. Dobrin et al. [2015] also found that wetting properties and wheat germination were improved after a non-thermal discharge plasma treatment. In this study, the wheat seed coat was softened after the DBD plasma treatment; these phenomena suggest that the wheat seed surface underwent oxidation by the DBD plasma, which might account for the increase in water uptake of the wheat seeds. Wild and Kesmodel [2001] reported that the chemical structure and roughness of the seed surface could be changed by a plasma treatment, which resulted in the change of wetting behavior of the seed and affected its water uptake. Because the hydrophily of the seeds was enhanced by the plasma treatment, its ability to absorb water would also be enhanced, leading to better growth [Jiang et al., 2014]. Vashisth and Nagarajan [2008] found that the physiological activity of chickpea seeds was improved after a static magnetic field treatment; they attributed this improvement to greater absorption of moisture, and the germination and seedling growth of the chickpea seeds was promoted.

The relative electroconductivity of seeds treated by DBD plasma was significantly increased in this study (T1–T4 treatments in Table 4), which could be explained as the etching effect of the DBD plasma that produced cracks on the seed coat in Figure 3d, resulting in water uptake improvement and seedling growth acceleration. Similar phenomena were observed by Tong et al. [2014], who found that the relative electroconductivity of seeds was enhanced after an air plasma treatment; they attributed this result to the formation of tiny holes in the seed coat because of the etching effect of air plasma. On the contrary, the DBD plasma treatment with a higher energy level had no significant influence on the relative electroconductivity of the wheat seed (T5 treatment in Table 4), and this might be due to the repositioning effect by the DBD plasma.

Soluble protein and α-amylase activity played significant roles in plant physiological reactivity. For example, soluble protein played a significant role in plant growth and was quite an important component of numerous plant enzymes [Berry and Downton, 1982]. The plasma treatment could lead to a deeper penetration of plasma-induced UV radiation and reactive species (including ions, reactive oxygen species, and reactive nitrogen species) inside the seed, which could further favor certain biochemical reactions [Grzegorzewski et al., 2010]. Li et al. [2014] reported that the cold plasma treatment could enhance soluble protein content. In this study, the soluble protein content and α-amylase activity of wheat seedlings were both improved after the DBD plasma treatment compared with those of the control (T2 and T3 treatments in Table 4). These results were similar with the findings of Wu et al. [2007], who reported that a cold plasma treatment improved the soluble protein content of corn seedlings. Yin et al. [2005] also observed that soluble protein content and α-amylase activity of tomato seedlings were enhanced by the cold plasma treatment. These results suggested that a DBD plasma treatment might be able to induce the activation of physiological reactivity in wheat seed, which then stimulates germination and growth.

CONCLUSIONS

The behavior of wheat seed germination and seedling growth exposed to a DBD plasma system at atmospheric pressure and room temperature was investigated in this study. Germination potential, germination rate, germination index, and vigor index were all improved after the DBD plasma treatment at appropriate energy levels, as well as seedlings' length and weight. The DBD plasma treatment resulted in the oxidation of the wheat seed coat. The wheat seed coat was softened and cracks occurred on its surface, which led to the improvement of water uptake and the permeability of the seeds, subsequently benefiting its germination. The active species penetrated into the wheat seed caryopses and activated their physiological reactivity, resulting in soluble protein and α-amylase activity enhancement.

DBD plasma not only affected wheat seed surface modifications but was also involved in several physiological reactions inside the seed, which were reflected in changes in the germination and growth of the wheat seeds. Therefore, DBD plasma might be an efficient and viable approach for wheat seed improvement.

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