Improvement of Honeydew Seed Germination Using Dielectric Barrier Discharge Planar (DBDP) Cold Plasma

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Abstract. Cold plasma treatment has been widely applied to enhance the growth of plants, especially for research purposes. Due to its significant advantage of not using chemicals, the potential of using cold plasma in the agricultural sector has been proven to cause no harm to crops, humans, or the environment. This study is conducted to investigate the effectiveness of dielectric barrier discharge planar (DBDP) cold plasma treatment on seed germination and water absorption of honeydew seeds. The treatment was carried out using an air compressor with a flow rate of 100 sccm with a constant applied peak to peak voltage of 3.5 kV. The honeydew seed samples were then exposed to various treatment times (0, 120 sec, 150 sec, 180 sec, 210 sec, and 240 sec). For treatment analysis, the length of root and shoot for each seed was recorded on a daily basis for seven days. The contact angle was also measured to determine its hydrophilicity. The data obtained show that honeydew seeds treated with DBD cold plasma has 105.63% difference increment in seed germination and 59.20% different increment of water absorption.

INTRODUCTION

Cucumismelo L.inodorus is the scientific name for the honeydew fruit [1]. China first planted 16,009,584 tons of honeydew, followed by Turkey and Iran [2]. Bio-priming and seed film coating methods were used to increase honeydew production with high quality and early-ripening fruits. However, both of these methods have their constraints. Bio-priming is an alternative to chemical control, which results in infected or contaminated seeds. Besides, it cannot improve the rate and uniformity of seed emergence or reduce damping-off disease on its own [3]. On the other hand, the film coating method necessitates multiple polymer coatings, and the coating application must be stable under seed-handling conditions encountered in

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manufacturing, storage, distribution, and seeding [4]. Therefore, cold plasma treatment was introduced to increase honeydew production with high quality and early-ripening fruits. Cold plasma has been used for a wide range of crops for commercial use, which can operate at atmospheric pressure [5]. The oxidation process on the honeydew seed surface was stimulated during the cold plasma treatment, resulting in seed surface hydrophilization. Absorption of water causes growth enhancement on the seeds. The other factor that can improve seed germination is the impact of plasma on a spectrum of plant development and physiological processes [6].

Plasma is the fourth state of matter that is composed of neutral gas atoms/molecules, ions, electrons, and reactive species. Plasma can be generated by supplying sufficient amount of energy to the gas to induce ionization. Cold plasma is obtained under atmospheric pressure or reduced pressure (vacuum) and requires less power input. For these reasons, cold plasma has been used due to the high selectivity and energy efficiency of plasma chemical reactions. Cold plasma has the advantage where it can operate effectively at low temperatures and without quenching [7-9]. Plasma can interact with seed [10], rice [11], and mushroom spawn [12], which affect the seed surface characteristics. Plasma treatment can enhance the germination rate by removing microbial [13] and increasing water absorption on the seed surface [14]. Meng et al. (2017) showed the impact of using dielectric barrier discharge plasma on wheat seed [15]. Fadhlalmawla et al. (2019) focused on the effect of a cold atmospheric pressure plasma jet on seed germinations and fenugreek seed growth using two-electrode argon cold atmospheric pressure plasma jet (CAPPJ) system [16]. This study treated the honeydew seed for improvement of germination and seeds surface morphological characteristics by using the dielectric barrier discharge planar (DBDP) cold plasma. Thus, the germination process of the seeds and the seeds' hydrophilicity for water absorptions capability were measured in a laboratory scale.

MATERIALS AND METHODOLOGY

The DBDP-CP Setup

The experiment was carried out using a new design of dielectric barrier discharge planar (DBDP) cold plasma system. The rectangular planar DBDP reactor, with dimensions of 9 cm x 11 cm x 1 cm (w x l x t), was made by stacking Kapton tape (10 layers) on an acrylic plate as a dielectric material and copper foil tape as an electrode, both positioned parallel to each other. The 7 cm x 9 cmx 0.3 cm (w x l x t) of acrylic plate was punched into 5 holes using drilling machine to place the sample holder (2.5 cm each hole) so that a large number of seeds could be treated at one run treatment before being placed in the middle of the reactor. The samples were put in the sample holder respectively with 0.3 cm discharge gap. The schematic diagram of the DBDP system for seed treatment is illustrated in Figure 1. The DBDP was set in a fume chamber. Using an air compressor (FD 1006 A, 1HP), the gas with 100 sccm of flow rate was flown into the DBDP system, by considering different time exposure (0 sec, 120 sec, 150 sec, 180 sec, 210 sec, and 240 sec) respectively. DBDP was generated by supplying 3.5 kV AC peak to peak high voltage.

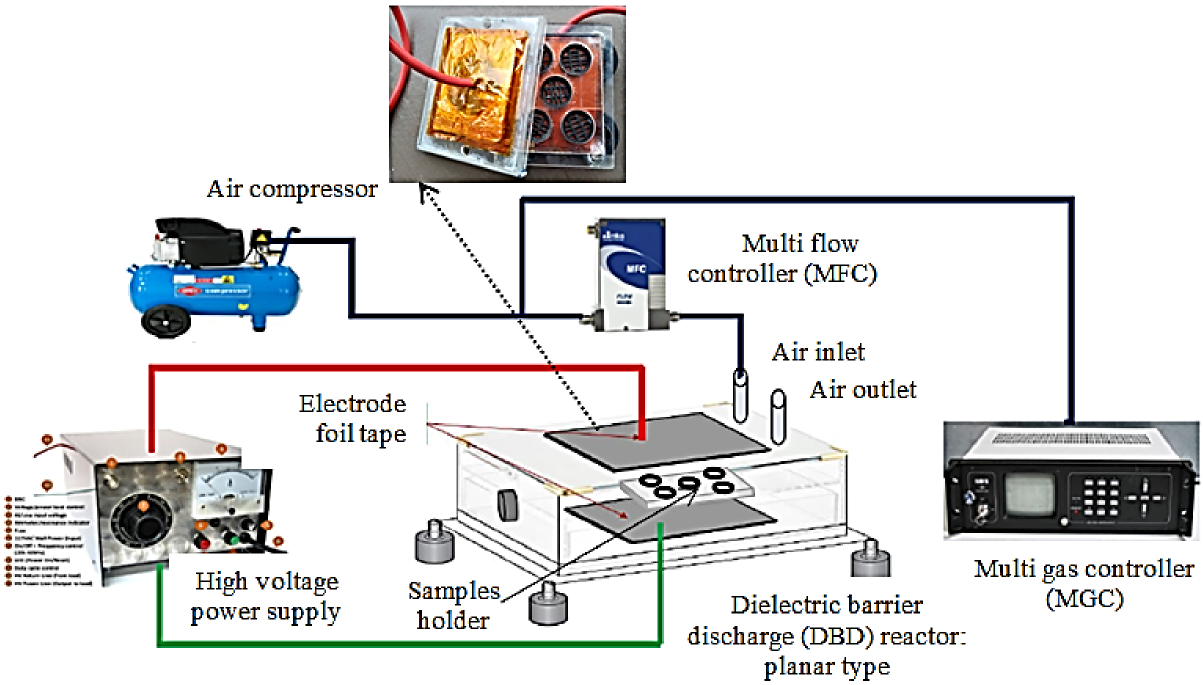

FIGURE 1. The schematic diagram of the DBD-CP system for seeds treatments.

The Germination Process

The honeydew seeds were obtained from market. All collected seeds were air-dried until totally dry. Only 42 quality seeds were selected. Seed germination tests were conducted with seven replicates of DBDP-treated and untreated seeds, respectively. The treated seeds were placed in a container containing one layer of tissue and two-layers of cotton, and each container contained seven seeds. The samples were watered with 2 ml of tap water twice a day, stored at 25 ºC in the light/dark (12 h/12 h) place and observed for germination growth. The daily record of germinated seeds was taken within 7 days using centimeter scale ruler.

The Contact Angle Measurements

The optical contact angle measuring and contour analysis system (OCA) was used for contact angle measurement. The contact angle between the seed surface and liquid (distilled water) was measured at room temperature by placing a drop of 1 µl of liquid on the seed surface. The OCA device combines high-resolution optics, exact liquid dosing, and precise sample positioning for a reliable measuring system.

RESULTS AND DISCUSSIONS

The Seeds Germinations

Table 1 highlights the depths of untreated and treated honeydew seeds germinations for seven (7) consecutive days. On day 3, the seeds for 120 sec and 150 sec started to grow, indicating that plasma treatment had a significant stimulatory effect on seed germinations, whereas other treatment times showed growth on day 4. The seeds treated with plasma up to 150 sec showed the longest root to a stem length of 14.6 cm compared with untreated seeds which were only 7.1 cm in length. Nonetheless, the treatment with the longest duration (240 sec) has the shortest length (1.8 cm). The data gathered showed that the cold plasma has a tendency to open the pores of the seed and increase the water absorption, besides enhancing germinations. However, plasma treatment with longer time treatment (240 sec) might be too strong that can affect the seed properties, slowing the germination process of the honeydew seeds. In line with this, treatment in short period also causes the seeds to not get sufficient energy and plasma species during the oxidation process, though this is not effective for the germination process. Zhang & Yan (2018) also found that plasma treatment improves seeds' vigor and promotes seed germination and seedling growth. It also enhances the seedling desiccation tolerance, water holding capacity and improves drought resistance [17]. Agun et al. (2020) treated myelium mushroom using plasma treatment and found that the cold plasma influences mushroom germination and production [12]. According to Mahdavian & Koocheki (2020), cold plasma treatment also yielded better surface charge and protein solubility of protein isolate on grass pea (*Lathyrussativus L.*), increasing the cold plasma treatment time [18]. For the time being, the effect of 150 sec plasma treatment and 3.5 kV voltage is satisfactory to yield apparent effect with 105.63% on the honeydew seed as calculated by using Equation (1).

TABLE 1. The measurements of the honeydew seeds germination from root to the leaf

Treatment time (sec)	Germination length (±0.1 cm)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
0	0	0	0	0.4	1.4	3.5	7.1
120	0	0	0.2	0.3	3.7	5.6	7.4
150	0	0	0.5	1.4	3.9	9.1	14.6
180	0	0	0	0.1	0.8	4.2	5.7
210	0	0	0	0.1	0.4	0.8	1.9
240	0	0	0	0.1	0.3	0.6	1.8

$$(\%)\boldsymbol{Germination\ increment\ for\ 150s} = \frac{\mathbf{14.6-7.1}}{\mathbf{7.1}}\boldsymbol{X}\ \mathbf{100} = \mathbf{105.63}\% \quad \textbf{(1)}$$

Figure 2 shows the qualitative results of the best germination of honeydew seeds on the fourth (4) and 7th days. The observation showed that the 150 sec treatment gave the fastest germination when clearly displaying longest roots steam length as can be seen in 4 days compared to 7 days germination. On day 7, treatment at 150 sec resulted in the growth of leaves compared to the untreated and other treatment times. This observation indicates that proper selection of DBDP treatment time is essential for the seeds to germinate better and faster. According to Szőke et al. (2018), the plasma glow provides some hope for seeds on the early stages of plant development in the field. This phenomenon occurred due to the high energy efficiency of plasma chemical reactions. When plasma was exposed to the seed surface, the reactive oxygen and nitrogen species interacted with the seed surface, removing microbial and changing the seed properties. This mechanism increases water absorption on the seed surface and promotes seed germination and seedling growth [17,19].

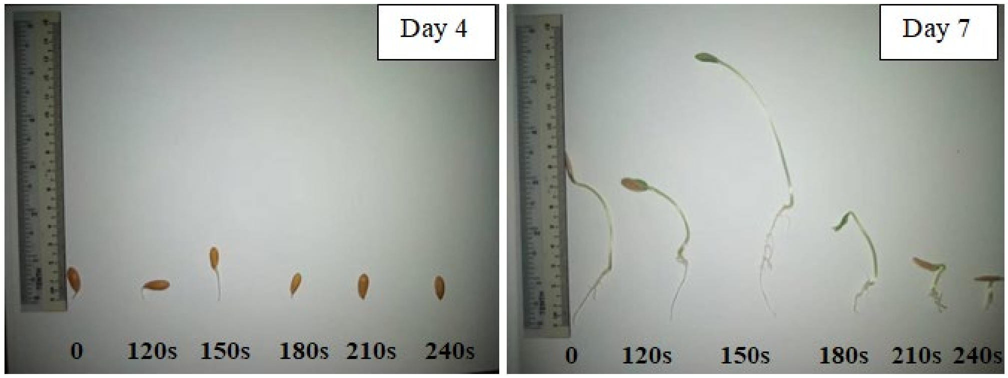

FIGURE 2. The total length of the roots and steam after germination of the treated and untreated seed up to day 4 and day 7

The Seeds Contact Angle

The result on the effect of DBDP cold plasma treatment on hydrophilicity of the seeds is depicted in Figure 3 by the apparent contact angle between the tangent to the liquid-air interface and the apparent solid surface macroscopically. The plasma treatment dramatically decreased the apparent contact angle in the samples compared to the control group. It was found that the contact angle for the untreated, 120 sec and 150 sec of cold plasma treatment honeydew seeds were 64.38º, 56.41º and 26.27º respectively. This indicates that 150 sec has good water absorption because due to its smaller contact angle, making the surface more hydrophilic with 59.20% contact angle decrement as calculated in Equation 2. The surface etching caused by the plasma species increased the seeds coating conductivity, further increasing its hydrophilic characteristic. This proves that 150 sec of cold plasma treatment yielded strong wettability seeds which can absorb more water to stimulate seed germination. With the increase in plasma treatment time, the contact angle is further decreased. According to Sadhu et al. (2017), the contact angle decreased by a maximum of 57.0% in 20 min treated sample compared with the control group for mung beans. Plasma etching increases surface area, which is responsible for the hydrophilicity, resulting in increased water imbibition of treated samples when compared to untreated samples. The cold plasma treatment significantly decreased the contact angle of grains [20]. Volkov et al. (2019) also found that treating pumpkin seeds with cold atmospheric pressure plasma can reduce the apparent contact angle between a water drop and a seed surface while also increasing the wetting properties of seed surfaces [21].

FIGURE 3: Water droplet deposited on (a) untreated (a), 120 sec and (b) 150 sec on honey dew seeds

$$(\%)\textbf{Water absorption increment for 150s} = \frac{26.27-64.38}{64.38}\mathbf{X\ 100} = \mathbf{59.20}\% \quad \textbf{(2)}$$

CONCLUSION

In conclusion, the dielectric barrier discharge (DBDP) cold plasma is a promising technology for agricultural application. It opens up a new way to increase the level of agricultural production, especially for seed germinations. The key findings show that DBDP cold plasma treatment with 150 sec treatment time and 3.5 kV of voltage is the best processing parameter for honeydew seed treatment because it stimulated 105.63% of honeydew growth and increased 59.20% of water absorption.

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