



Increased germination and growth rates of pea and Zucchini seed by FSG plasma

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Abstract

Recently, cold atmospheric plasma (CAP) with the unique bio-disinfection features is used in various fields of industry, medicine, and agriculture. The main objectives of this work were to design FSG plasma (a semi-automatic device) and investigate the effect of the cold plasma in the enhancement of the Pea and Zucchini seed germination. Plasma irradiation time was studied to obtain a proper condition for the germination enhancement of seeds. The growth rate was calculated by measuring length of root and stem and dry weight of plants treated by plasma. To investigate drought resistance of plants, all treated and untreated samples were kept in darkness without water for 48 h. From the experimental results, it could be confirmed both drought resistance and germination of seedlings increased after plasma was applied to seeds at 30 s, while seeds treated whiten 60 s showed a decrease in both germination rate and seedling growth.

Keywords FSG (fast seed growth) plasma · Germination rate · Drought resistance

Introduction

Plant's life begins from the seed. Hence, seed health, growth, and resistance to environmental factors are very important. Considering the daily increase world population, increasing demand for food and reduced water resources, and human has to find a way to increase food with lower cost. In order to deal with mentioned problems, increasing seed quality and increasing their resistance to environmental elements (bacteria, drought) can be useful. Seed germination begins when dormant embryo starts to grow. This phase for most seeds begins with imbibition of water. Usually, imbibition starts with water absorption (phase I), and then, the phase II which is a plateau phase and starts with some changes in the amount of water absorption, and a subsequent increase in water content with radicle growth (phase III). Regulation of water and other environmental factors may be critical for improving seed

germination and successive growth, and this should be considered in developing technologies for efficient germination and growth [1, 2]. Recently, studies show that cold atmospheric plasma systems could promote seed germination and growth [1, 3–15]. Increment seed germination rate, growth development, and crop yield by plasma treatment have been reported in various crops: spinach [16], wheat [8, 13], soybean [9], and radish [17]. Cold atmospheric plasma systems consist of positively and negatively charged particles (free electrons and ions), and neutral activated species including excited molecules, UV photons, long and short lived free radicals, reactive oxygen species, reactive nitrogen species, and some ozone [18]. Cold atmospheric plasma can penetrate into the capsule of the seeds, and the redox reaction inside plant cells induced by oxygen radicals may affect seed germination and growth.

The main aim of this study was to design and construction of semi-automatic cold plasma device and investigates the effect of air plasma on seed germination, seedling growth, and seedlings resistance against darkness and drought.

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Materials and methods

Seed material

Both Pea and Zucchini seeds were purchased from Bazram Company and only ripe intact seeds without visible defects were selected.

FSG plasma device

Self-designed and self-made FSG plasma is shown in Fig. 1. The system consists of high-voltage AC power supply, a gas flow regulator, and a plasma nozzle (gliding arc) with ability of producing 3 cm plasma. Air gas was used as the main plasma gas (5 L/min). The applied voltage was 15 kV, and the distance between the opening of the plasma nozzle and the samples was 1 cm for all cases of plasma treatment.

The mechanical part consists of a mechatronic arm with two degrees of freedom, which has three hinges that work with separate engine. The system controls by real time, and users can choose the device performance. The mechatronic performance of devices in such a way that, at first, the type of commands gives to device by communication panel, and then, they analyze by the main processor and then sent to the movement angle motor and the speed is determined by the Jacobian, and finally, the arm transfers the seed box, the location of the plasma. After treating with plasma, the arm returns the seeds to first chamber.

Microbial analysis

To investigate the effect of plasma exposure on microorganisms, some seeds from both the untreated and treated

sample were selected randomly, and then mixed with 100 mL of sterile peptone (0.1%) and were placed on the shaker for 6 h. After that, 50 μ L of each sample were spread onto Müller–Hilton agar media and incubated at 35 °C for overnight. In this study, five replicated samples were used from each treated and untreated sample. The survival curve of microorganism was expressed corresponding to the surface area (CFU/mL/cm²) of the seeds.

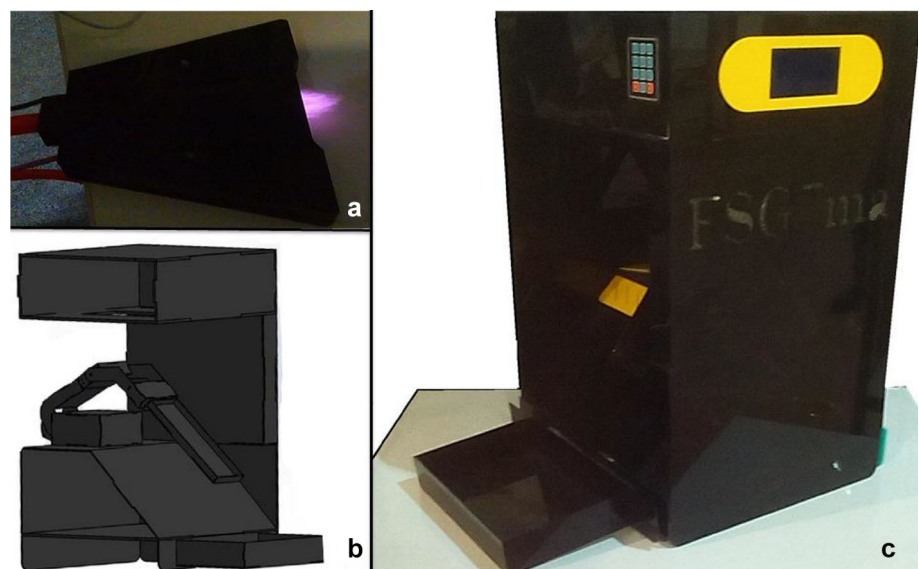
Seed preparation and plasma treatment

Thirty seeds were kept in sterile deionized water for 1 h. Then, five seeds were placed in the 60 mm petri dish which was covered by dried filter and exposed to the plasma. Plasma treatment was given at the intervals of 30 and 60 s. Gas treatment without plasma discharge was used as a control. After each treatment, treated seeds immediately were planted in pots with the same measure of the soil (40 g in each pot) and incubated in growth chamber (25 °C, 50% humidity, light for 16 h, and dark for 8 h) for 14 days. Each experiment included six replicates with five seeds per treatment. Seed germination was measured every 24 h by registering the emergence of the radicle and continued until no more seed germinated. Finally, all treated and untreated seeds were kept in darkness and drought for 48 h to investigate the resistance of plants against darkness and drought. The mean germination time (MGT) was calculated by Eq. 1 [19]:

$$\text{MGT} = \frac{\sum tm}{\sum t}, \quad (1)$$

where n is the number of germinated seeds after t days, counted from the beginning of germination.

Fig. 1 FSG plasma device: **a** plasma nozzle, **b** schematic of device, and **c** the image taken from main device



Equation 2 was used to determine the speed of germination (V_g), where n_t is the number of newly germinated seeds at time t , counted from the first day [20]:

$$V_g = \sum \left(\frac{n_t}{t} \right). \quad (2)$$

Ten seeds from both untreated and treated samples randomly selected and the root and shoot of them were measured after 7 days. Then, the selected seeds were dried at 90 °C; dry weight was measured.

Statistical analysis

The experimental data were expressed as the style of mean value \pm standard deviation (SD). The differences among means were tested for statistical significance by one-way analysis of variance and independent sample T test. The p value < 0.05 was defined as statistically significant.

Result

Plasma effect on the microorganisms

The antibacterial effect of plasma depicts in Fig. 2. The amount of microorganisms on both untreated Pea and Zucchini seeds was 5.5 ± 0.03 (CFU/mL/cm²) and treated with plasma for 30 and 60 s. According to the survival curve, the amount of microorganisms reduced significantly with increasing the plasma treatment times. Cold plasma contains various particles including, positively and negatively charged particles (free electrons and ions), some neutral activated species such as excited molecules, UV

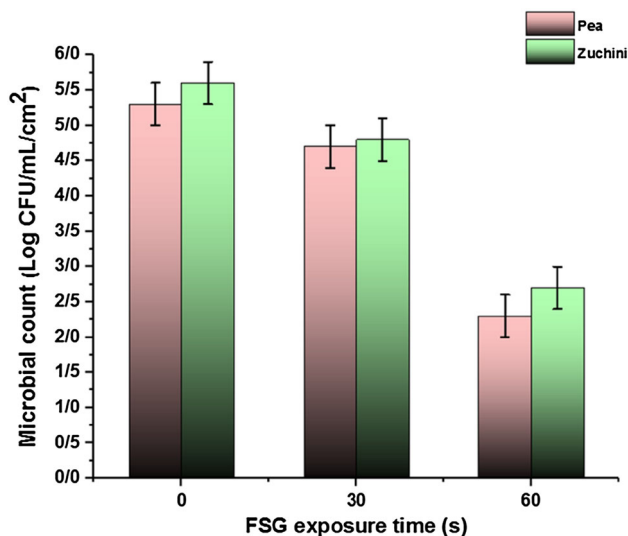


Fig. 2 Survival curve of the surface microorganisms on Pea and Zucchini due to FSG plasma exposure. Seed treatment indicates significant differences at $P < 0.05$ level among all treatments

photons, long and short lived free radicals, reactive oxygen species (ROS) such as O, O₂, O₃, and OH, reactive nitrogen species (RNS) such as NO, NO₂, NO_x, and ozone that can be used for disinfection of non-living surfaces (medical tools), living tissues, bio-decontamination, and sterilization of heat sensitive material [18]. Many studies have generated different types of cold atmospheric plasma (CAP) and tested their antibacterial activity [21]. It seems that the disinfection efficiency of CAP is related to the specific properties of the devices used and the type of bacteria. Studies have shown cold plasma is being able to effect on different targets within the cell including cell wall intracellular proteins and DNA [18]. Mechanical stress induced by charged species is able to break important bonds in the cell structure [22]. Therefore, this disruption of the outer shell of the cell will lead to leakage of cellular components such as proteins and potassium nucleic acid. After disruption of cell wall structure, reactive species can penetrate into the interior of the cell and further damage DNA and intracellular protein from oxidative and nitrosative species [18]. It should be mentioned here that both unsaturated fatty acids in the membrane lipids and protein molecules imbedded in the lipid bilayer can be susceptible to attacks by these reactive species [21].

Seed germination characteristics

The effect of FSG with different exposure times on the Pea seeds germination potential is given in Table 1. Pea seeds were treated by cold plasma germinated in the first day. After 10 h, the treated Pea seeds with 30 s plasma exposure germinated, while the seeds which were treated at 60 s plasma exposure germinated after 15 h and untreated Pea seeds germinated after 24 h. A significant difference achieved between untreated and treated samples.

The highest percentage of germination is related to the 30 s plasma exposure (80.4%). For samples which were irradiated by plasma for 30 s, the speed of germination (V_g), the length of shoot and root, the germination rate, and the seedling dry weight increased (Table 1).

Germination of Pea seeds was more rapidly increased after treatment with 30 s high-voltage plasma. After 14 days, about 80 and 74% of seeds were germinated at 30 and 60 s treatment, while just 40% of untreated seeds were germinated. After 14 days, the length of seedlings which were treated by plasma at 30 s was more than other samples. Figure 3 shows the image of Pea seeds after 7 and 14 days (Fig. 3b, c). As is clearly in images, the maximum growth of shoot and root related to the seeds was treated at 30 s by plasma exposure.

Table 2 shows the results obtained from Zucchini seeds. The same results with Pea seed were achieved. The most amount of seed germination and growth rate is related to

Table 1 Germination specifications of obtained from Pea seeds at different FSG plasma exposure times

Parameter treatment time (s)	GEM (%)	MGT (day)	Vg (seed/day)	Seedling length (cm)	Seedling dry weight (mg)
Control	40	2.8	3.4 ± 0.1	12.5 ± 0.3	23 ± 1
30	80.4	3.5	7.2 ± 0.1	23.3 ± 0.3	52 ± 3
60	64.6	2.7	5.3 ± 0.1	19.2 ± 0.3	29 ± 2

Fig. 3 Image of pea seeds: **a** workplace, **b** after 7 days, and **c** after 14 days**Table 2** Germination specifications obtained from Zucchini seeds at different FSG plasma exposure times

Parameter treatment time (s)	GEM (%)	MGT (day)	Vg (seed/day)	Seedling length (cm)	Seedling dry weight (mg)
Control	38	2.8	3.6 ± 0.1	18.1 ± 0.3	32 ± 2
30	72.5	4.2	8.3 ± 0.1	27.3 ± 0.3	67 ± 1
60	50	2.8	4.5 ± 0.1	24.4 ± 0.3	59 ± 1

the seed which were treated at 30 s plasma exposure. However, the total results were less than Pea seeds, so that just 72.5% and 50% of Zucchini seeds germinated after treatment at 30 and 60 s plasma exposure, respectively, while, similarly about 40% of untreated seeds germinated. The treated Zucchini seeds at 30 s germinated after 48 h of plasma exposure, while the untreated Zucchini seeds germinated after 5 days. In contrast, the MGT of Zucchini seeds were more than pea seeds (Table 2).

The images of zucchini seeds are shown in Fig. 4. After 7 days, just the root of untreated Zucchini seeds grew, while the whole length of treated seeds at 30 s plasma exposure was about 16 cm (Fig. 4b).

According to the results, some physiological effect decreased after 60 s of plasma exposure in both seeds. Several factors can be effective. According to the previous reports, some reactive species such as reactive oxygen and nitrogen play a crucial role in the adjustment of abscisic acid (ABA) catabolism and gibberellin (GA) biosynthesis during seed imbibition [23]. GA plays an important role in seed stem elongation, leaf expansion, germination, and seed development processes, while ABA has a significant effect in some physiological processes such as seed maturation, growth, and adaptive responses to environmental stresses, and dormancy [24].

Fig. 4 Image of Zucchini seeds, **a** workplace, **b** after 7 days, and **c** after 14 days



The growth rate under stress condition

Keeping plants under water and light stress conditions showed that seedlings from untreated seeds withered severely after 48 h, while the seedlings grown from treated seeds had more resistance to the stress. The treated seeds at 30 s plasma, under water and light stress condition after 48 h had more resistance than treated samples at 60 s plasma exposure. In the other experiment, similarly, Ling et al. [25] reported that seed treatment by cold plasma enhanced the germination rate even in drought stress conditions by increasing the absorptive ability attributed to seed imbibition.

Conclusion

In this study, we made self-design and self-made FSG plasma. The effect of plasma exposure on germination and growth rate and microorganism on seeds surface was investigated. A significant reduction of the seed borne microbial contamination was observed by increasing plasma exposure time. Moreover, increased seed germination was achieved with treatment times at 30 s for both Pea and Zucchini seeds. It is clear that plasma can increase the speed of plant growth. Hence, we can conclude that the FSG plasma has ability to increase the seed germination and plant resistance against drought and darkness.

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