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The impact of plasma treatment on the early growth stages of radish seeds

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The climate change has been posing the challenge in front of scientists to establish the alternative approaches in agriculture. In the last decade the food security issue became one of the central problems to end the extreme poverty, hunger and malnutrition. As a sustainable method plasma agriculture came into play. The irradiation of seeds by cold atmospheric plasma (CAP) resulted in improved wettability, accelerated germination time and growth processes. To better understand CAP effects to the radish seeds we performed in vitro experiments and investigated germination index, seedling growth and dry weight of radish sprouts. The outcome showed the positive data on CAP exposed seeds in comparison to the control case.

I. INTRODUCTION

The global demand for agricultural products is increasing day by day[1]. In order to supply the current need, basically chemicals are widely used on the field[2]. Apparently, over usage of pesticides degrade the soil condition[3]. Moreover, various types of chemicals have been found on vegetables and fruit which causing severe health issues around the world[4, 5]. Therefore, the main priority is to apply ecologically friendly technologies in agriculture. One of these kinds of technology is a cold atmospheric plasma (CAP) that has been offering many benefits without any adverse effects[6, 7].

CAP is a fourth state of matter which can be generated by applying high voltage between electrodes[8]. The irradiation of seed by different types of CAP devices resulted in bacterial decontamination, improved water uptake, enhanced the germination rate and stimulated growth processes of plants[9, 10]. It should be noted that, in turn, this possibly lead to save water spent on plants and to harvest the crop before its due date. According to the literature, CAP treated seeds overexpressed antioxidant proteins such as catalase, superoxide dismutase and peroxidase[11, 12]. Hence, CAP exposed seeds become also resistant against the plant diseases[13, 14]. Besides, seed development stages also varies depending on the employed plasma devices. Overall, the application CAP technology in agriculture can be economically and ecologically beneficial. The primary objective is to determine the favorable CAP treatment time of chosen seed to establish better protocols.

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Here, we studied germination index, seedling growth and dry weight of radish sprouts treated by dielectric barrier discharge (DBD). Our findings on plasma exposed seeds showed better results in comparison to control one.

II. METHODS AND MATERIALS

In this study, radish seeds (*Raphanus sativus L.*) with a shelf life of 4 years were chosen. 240 seeds of the same size, relatively similar size, weight and color are separated and further used in experiments. The seeds were treated for 180 seconds by placing them close to the electrodes. The detailed position of seeds shown in the figure below (Figure 1).

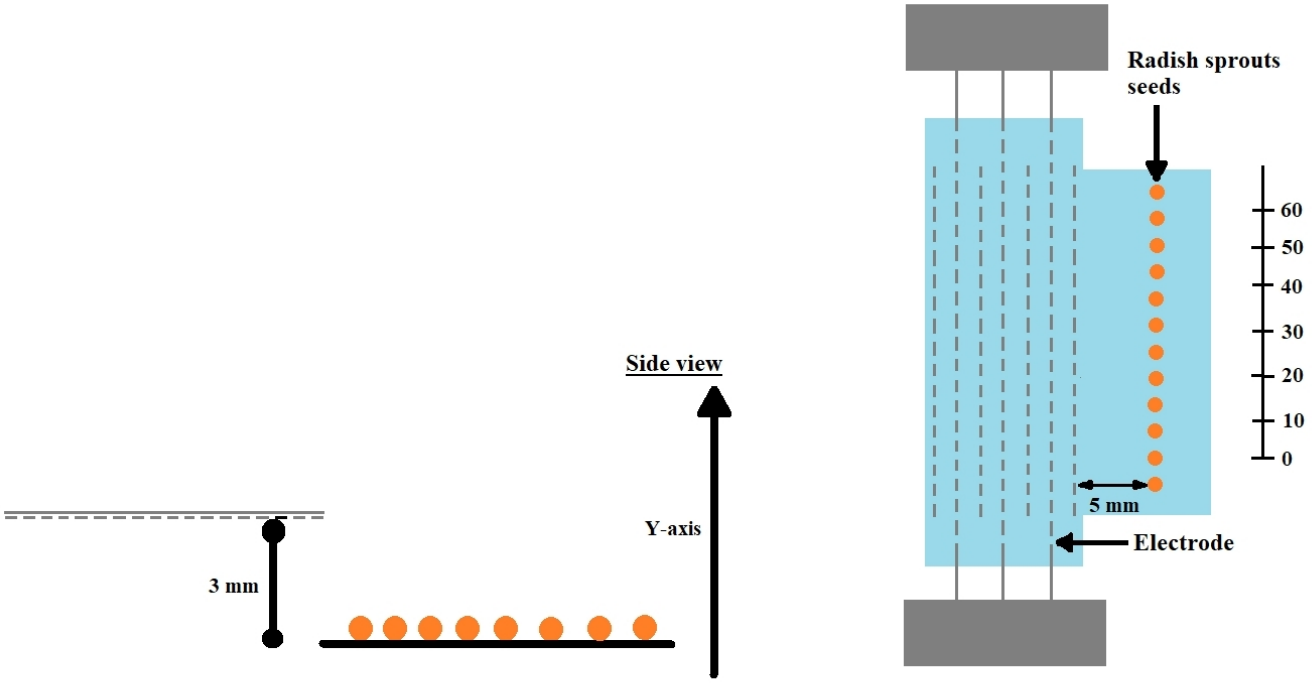


FIG. 1. *The schematic representation of DBD electrode and seed location.*

Half of the chosen seeds, i.e., 120 copies were treated with DBD. 10 seeds were used in each treatment time. Note that, DBD reactor also generates an electric field. Therefore, the seeds were placed at a distance of 5 mm away from the electrodes to avoid any possible electric field effect on the germination or seedling growth. The air circulation was arranged in such way that CAP generated RONS move towards to the seeds. The rest of the selected seeds were employed for the control case.

Plasma-exposed and control seeds were placed in four rows in a special container, and the bottom of the container was filled with ultra-pure, i.e., deionized water. Then, the seeds were moistened by spraying water and the container was covered with a thin polymer film (Figure 2b and 3a). Four holes were made with a diameter of 2 mm on polymer film. In the next step, it was covered with aluminum foil and placed in the storage chamber with temperature set of 24.3^0C (Figure 3b). Overall, three containers were used for control and another three containers were employed for plasma treated seeds.

III. RESULTS

In order to estimate the germination rate of seeds, containers were monitored in every 24 hours within 72 hours and the number of germinated seeds was determined. As is clear from the Table 1 that 4 to 5 control seeds were not germinated within 24 hours. In contrast, three seeds in container 1 and only one seed in container 2 were not germinated, while 100% germinated was observed in container 3. Both control and plasma irradiated seeds were fully

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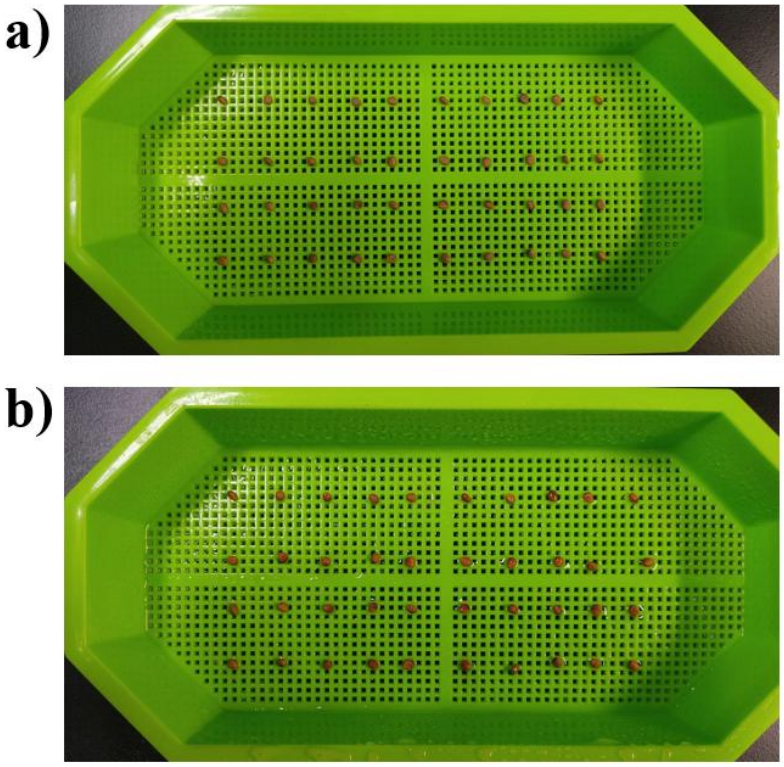


FIG. 2. *a) Placement of seeds in a special container. b) Seeds moistened with water.*

germinated after $2^n d$ and $3^r d$ day. Apparently, DBD exposure induced changes on seeds and it resulted in acceleration of germination rate in comparison to control seeds.

TABLE I. The number of germinated seeds monitored in every 24 hours.

The times when the study was conducted		container 1	container 2	container 3
24 hours	control	36	35	36
	plasma-treated	37	39	40
24 hours	control	40	40	40
	plasma-treated	40	40	40
24 hours	control	40	40	40
	plasma-treated	40	40	40

Further, the sprouts were analyzed after 72 hours. The sprouts were taken out of the containers and divided in two parts: stem and root. The collected both stems and roots were placed on board to measure the length (see Figure 4).

The length of the stems and roots calculated for each container (see Table 2). The results showed that plasma treatment influenced to the seeds. It consequently lead to increase of both stem and root length.

TABLE II. The average length of root and stem of plasma-treated versus control seeds.

	container 1 (mm)		container 2 (mm)		container 3 (mm)	
	root	stem	root	stem	root	stem
plasma-treated	24.0±6.50	14.72±4.40	27.58±7.60	14.08±3.60	19.88±8.58	12.55±3.70
control	17.44±6.78	11.60±3.57	23.12±8.92	10.40±3.9	17.35±6.07	10.60±3.87

We can observe that the length of the root and the primary stem from the plasma-treated seeds increased by 3 mm on average compared to the control (control: root - 19.32±7.75 mm, stem - 10.87±3.77 mm; plasma irradiated seeds: root - 23.82±8.16, stem - 13.78±3.97mm). The standard deviation error value is high. This is due to the less number of seeds considered in experiments and this can be reduced by performing experiments with more seeds. In

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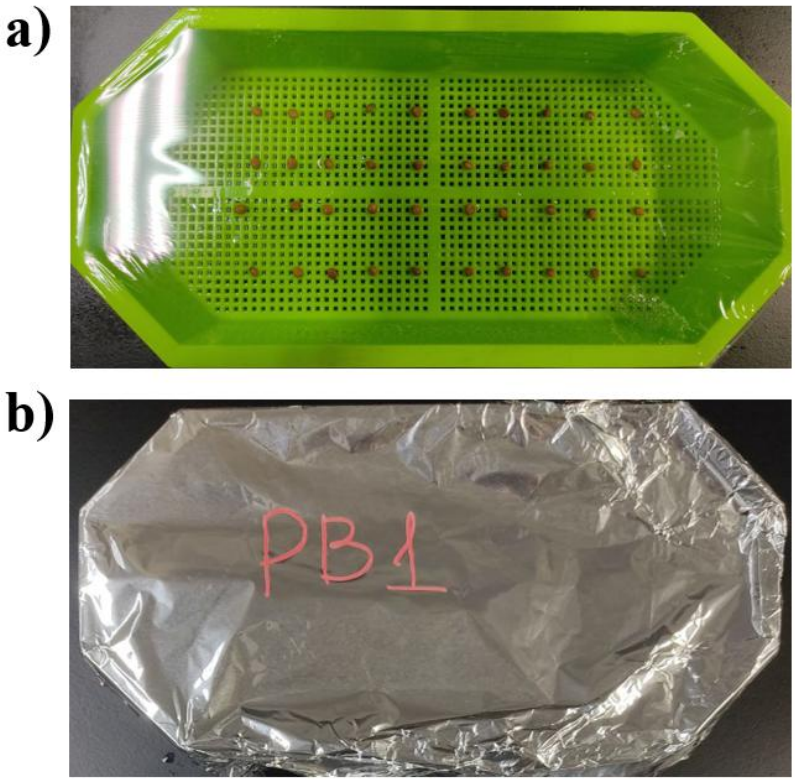


FIG. 3. *a) The seed container is covered with a polymer film b) Aluminum foil is covered over the film.*

addition, the sprouts collected after the measuring the stem and root length and were dried at 50^0C temperature for 24 hours by placing them in the storage chamber. The dry weight of the plasma treated sprout was 1.930 g, and the control was 1.809 g. Overall, plasma treatment also affected the dry weight of sprouts. Plasma devices produces both reactive oxygen and nitrogen species (RONS) and it is believed that these species play the primary role in physiology of seeds[6]. Due to the complexity of plasma predicting the certain mechanism is quite challenging. For instance, permeation of RONS to the water phase[15] and penetration of these species across the membrane[16–18] and aquaporin channels[19] have been studies in molecular scale. RONS reached the cell interior oxidize and nitrosylate proteins as well[20]. However, specifically RONS which induce growth stimulation is still unknown and requires more fundamental insight to obtain the complete picture in plasma agriculture field.

IV. CONCLUSION

In conclusion, the efficacy of DBD device in ambient air is examined by treating radish seeds. DBD generated RONS positively affected and accelerated the germination time compared to control seeds. This, in turn, also influenced the growth processes. It was determined that the length of the root and stem increased by 3 mm compared to the control during 72 hours. In addition, it should be noted that the dry weight of plant increased after plasma treatment compared to the control. In order to obtain substantial results we need to continue the experiment on the field by analyzing harvest on real conditions.

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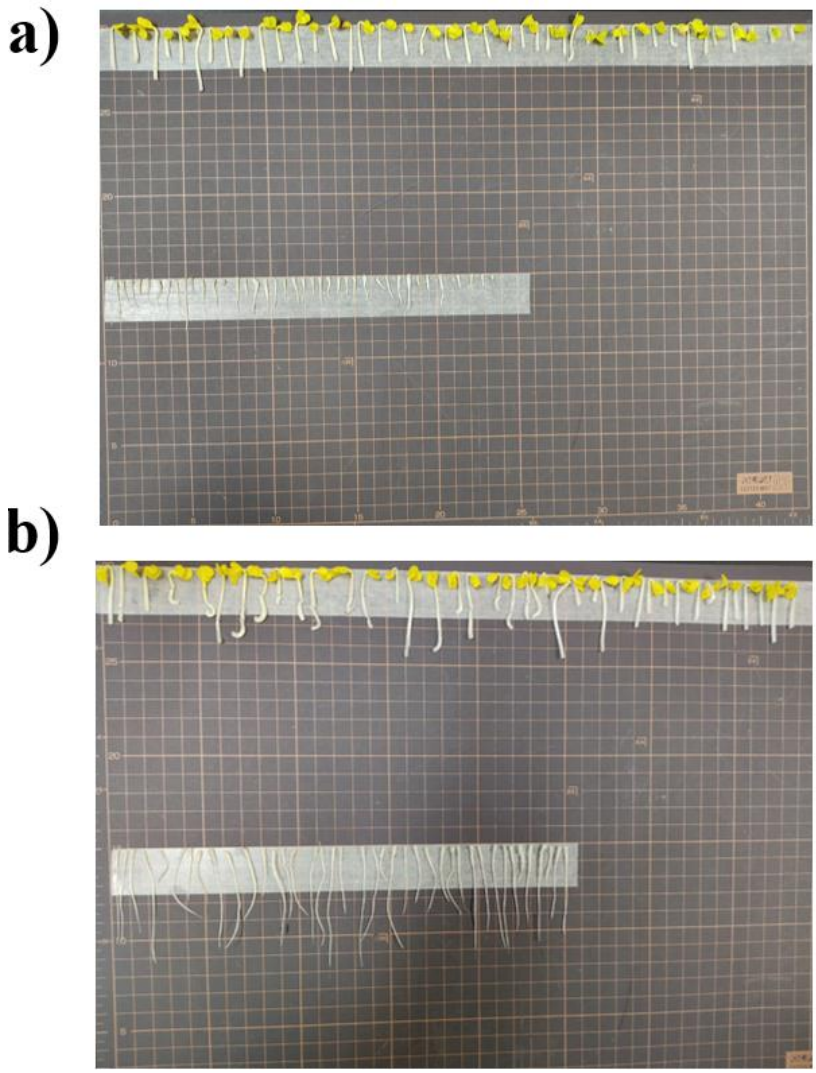


FIG. 4. Radish sprout's stem and root after 72 hours of preserving in containers: a) control and b) plasma exposed sprouts.

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