Growth Enhancement of Radish Sprouts Induced by Low Pressure O_2 Radio Frequency Discharge Plasma Irradiation

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Growth Enhancement of Radish Sprouts Induced by Low Pressure O_2 Radio Frequency Discharge Plasma Irradiation

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We studied growth enhancement of radish sprouts (*Raphanus sativus* L.) induced by low pressure O_2 radio frequency (RF) discharge plasma irradiation. The average length of radish sprouts cultivated for 7 days after O_2 plasma irradiation is 30–60% greater than that without irradiation. O_2 plasma irradiation does not affect seed germination. The experimental results reveal that oxygen related radicals strongly enhance growth, whereas ions and photons do not.

1. Introduction

Atmospheric pressure non-equilibrium discharge plasmas and low-pressure discharge plasmas have been widely employed in biomedical applications such as sterilization of microorganisms and surface coating of biomaterials because such plasmas induce little thermal damage to biomaterials.[1–8] There have been four distinct stages in the development of biomedical applications of plasmas (in chronological order): coating medical devices using discharge plasmas, inactivation of bacillus, direct plasma treatment of the human body to heal wounds, and cell growth regulation. One agricultural application in the fourth stage is the plasma treatment of crops by using pulsed power discharges and low-pressure discharges to enhance the growth of plants.[9–18] However, the growth enhancement mechanism has not been clarified because previous experiments contained many effects due to electric and magnetic fields, photons, ions, electrons, and radicals in the plasmas. Many studies have investigated growth enhancement induced by electrolyzed solutions,[19] methyl jasmonate,[20] and DC electric fields,[21] but such studies did not use plasmas and thus provide little insight into the growth enhancement mechanism for plasma irradiation. In this paper, we report experimental results on growth enhancement of radish sprouts by low pressure O_2 discharge plasma irradiation and we discuss the species that are important for growth enhancement.

2. Experimental Procedure

Experiments on the growth enhancement of radish sprouts were performed using a capacitively coupled RF discharge reactor (diameter: 200 mm; length: 450 mm). The discharge electrode was a single-turn stainless-steel wire (diameter: 4 mm; total length: 1800 mm). It was placed about 50 mm beneath the upper wall of the reactor (see Fig. 1). Pure O_2 or N_2 was supplied from the top of the reactor and the total pressure was 100 Pa. An RF voltage with a frequency of 13.56 MHz was applied to the powered electrode. The discharge power was 50 W. Optical emission spectra were obtained from the RF plasma using a spectrometer (Soma Optics S-2630) to obtain information on the radicals generated in the plasma.

We employed seeds of radish sprouts (*Raphanus sativus* L.) in the plasma irradiation experiments. For each irradiation condition, 20 seeds were used. The seeds were placed 150 mm below the top of the powered electrode and were spread on a cell culture dish (diameter: 35 mm). The plasma irradiation time was 30–120 min. After plasma irradiation, the seeds were cultivated in a plant incubator at 22 °C and 60% relative humidity in the dark with a pure water feed. Their weights and total lengths (i.e., from the root to the top of the stalk) were measured after cultivation for 3, 5, and 7 days using an electric balance and an image analysis system, respectively. The results obtained by the plasma irradiation were compared with results for controls by determining the statistical significance using the two-sided Student t-test.[22] Seed husks were observed by scanning electron microscopy (SEM; Hitachi S-3400N). The chemical bonds on the surfaces of the seeds were investigated using a Fourier-transform infrared (FTIR) spectrometer (JASCO FT/IR-4200) in attenuated total reflectance (ATR) mode.

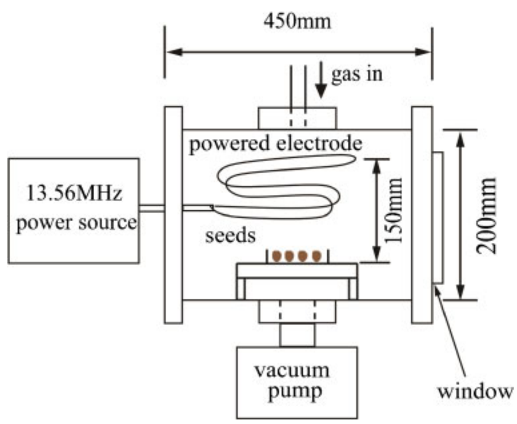

Fig. 1. (Color online) Discharge plasma reactor used to investigate growth enhancement of radish sprouts.

3. Results and Discussion

3.1 Growth enhancement of radish sprouts

Figure 2 shows typical images of radish sprouts after 7 days of cultivation for 30 min of O_2 plasma irradiation and without irradiation (control). All the seeds germinated within 12 h irrespective of whether they had been irradiated by plasma or not, which indicates that O_2 plasma irradiation does not affect seed germination. Radish sprouts that had been irradiated by the plasma were longer (from the root to the top of the stalk) than those that had not been irradiated.

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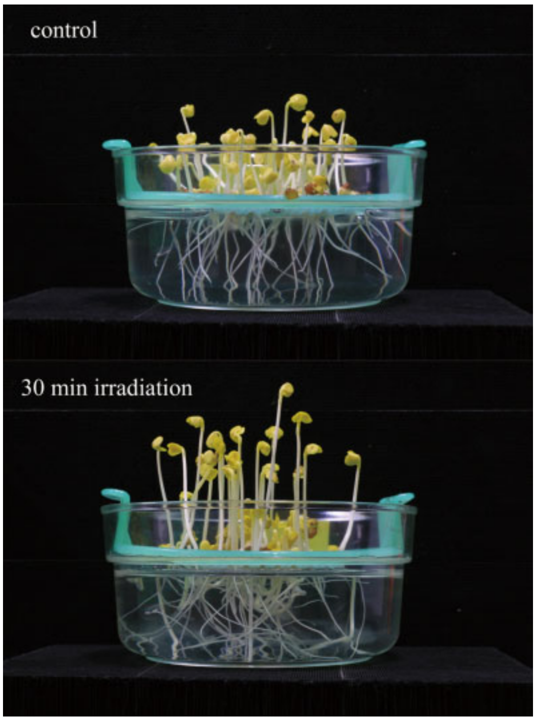

Fig. 2. (Color online) Typical images of radish sprouts cultivated for 7 days with and without O_2 plasma irradiation.

Figure 3(a) shows the average length of sprouts after cultivation for 7 days that had been irradiated by an O_2 plasma for 30 min, by a N_2 plasma for 30 min, and that had not been irradiated (the control). The average length of sprouts that had been irradiated by an O_2 plasma is 60% greater than that of sprouts that had not been irradiated for all discharge periods. In contrast, the average length of sprouts that been irradiated by a N_2 plasma is nearly the same as that of the control. We statistically analyzed these experimental results by using the Student t-test to compare the lengths of sprouts that had been subjected to plasma irradiation with those that had not. We used a significance level of $\alpha = 0.05$ for which the significance probability is less than 0.01 ($P < 0.01$). For 30–120 min irradiation, the sprout length has a large standard deviation. We used one-way analysis of variance with a significance level of $\alpha = 0.05$ to investigate whether there were significant differences between the average lengths of sprouts that had been irradiated for different times. We found that there were no significant differences between different irradiation times. Thus, we conclude that O_2 plasma irradiation enhanced the growth of radish sprouts, whereas N_2 plasma irradiation had little effect.

Figures 3(b) and 3(c) respectively show the dependences of the length and weight of radish sprouts on the cultivation period. Three days after irradiation, the length and the weight of sprouts that had been irradiated by an O_2 plasma are greater than those of sprouts that had not been irradiated; the difference in length is more pronounced than that in weight. Further study is required to clarify the mechanisms responsible for these differences and the dependence of the growth enhancement on the plasma irradiation time.

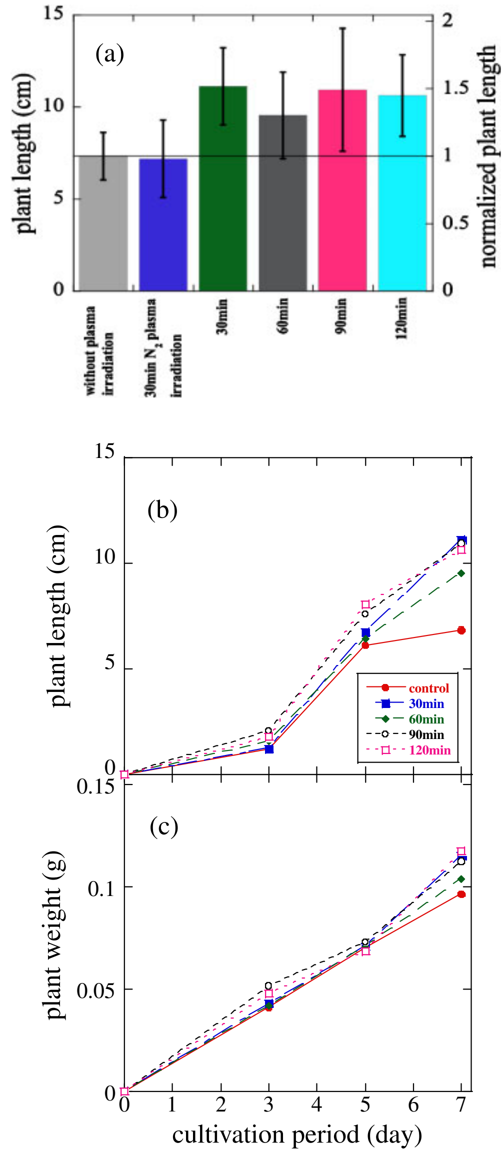

Fig. 3. (Color online) (a) Length of radish sprouts cultivated for 7 days after N_2 plasma irradiation, after O_2 plasma irradiation and the control. Time evolution of (b) length and (c) weight of radish sprouts after O_2 plasma irradiation. Error bars indicate the standard deviation of the length for each condition.

Figure 4 shows a typical plasma emission spectrum obtained 150 mm below the top of the powered electrode (i.e., the position where the seeds are irradiated by the plasma). The atomic oxygen emission lines, namely the 3p ^{5}P $\rightarrow$ 3s ^{5}S transition of O at 777.1 nm and the 3p ^{3}P $\rightarrow$ 3s ^{3}S transition of O at 845 nm,[23] and the emission line of the $b^1\Sigma_g{}^+ \rightarrow X^3\Sigma_g{}^-$ transition of O_2 at 762 nm[24] are clearly visible. These results suggest that seeds are exposed to oxygen radicals, such as excited states of O_2 and the ground and excited states of O.

3.2 Growth enhancement mechanism

There are three possible mechanisms for the growth enhancement induced by O_2 plasma irradiation: (i) oxygen radicals, such as excited states of O_2 and the ground and excited states of O, induce oxidation reactions in the cell metabolic cycle; (ii) photons and/or ions induce chemical reactions in cells;[25] and (iii) etching of the seed husks

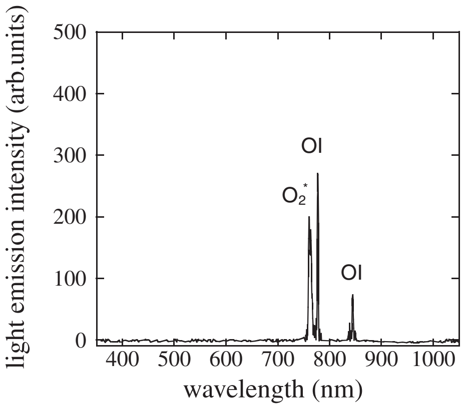

Fig. 4. Typical emission spectrum measured 150 mm below the top of the powered electrode for O_2 plasma irradiation (100 Pa; 50 W).

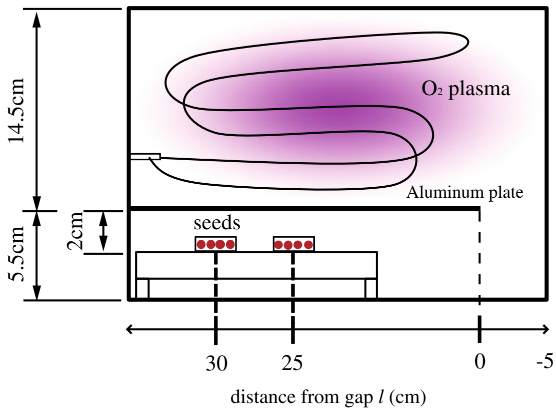

Fig. 5. (Color online) Schematic of reactor with an aluminum plate that suppresses irradiation of seeds by ions and photons.

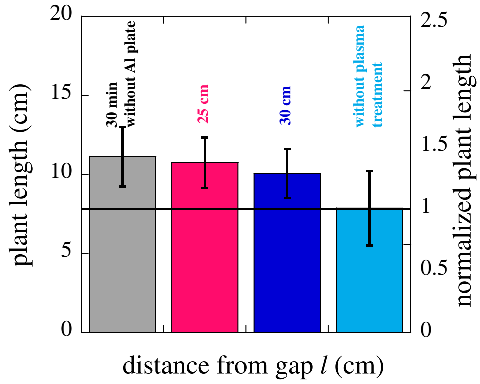

Fig. 6. (Color online) Lengths of radish sprouts after 7 days of cultivation after O_2 plasma irradiation and control using reactor with an aluminum plate. The error bars indicate the standard deviation of the length for each condition.

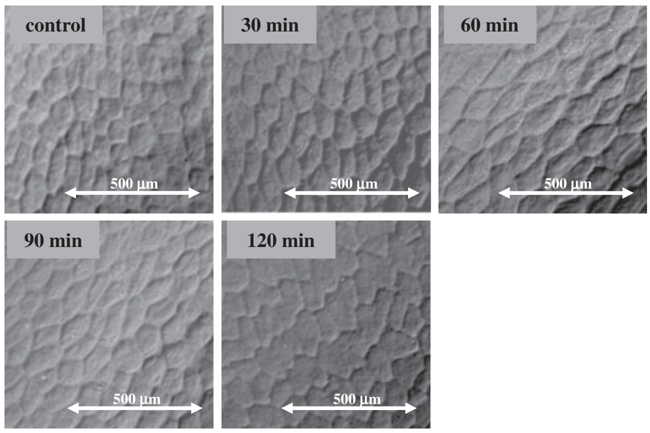

Fig. 7. SEM images of seed surfaces for various O_2 plasma irradiation times.

stimulates germination. To investigate the effects of photons and ions on growth enhancement, we placed an aluminum plate between the discharge electrode and the seeds to prevent photons and ions from impinging on the seeds (see Fig. 5). The aluminum plate did not extend across the entire length of the reactor; rather there was a 5 cm gap between the end of the aluminum plate and the reactor wall at one end of the reactor. Seeds were placed at distances of $l = 25$ and 30 cm from the end of the aluminum plate. Oxygen radicals could diffuse from the discharge region to the seeds via the gap, whereas most photons and ions from the discharge were blocked by the plate. The oxygen radical flux at the seeds is expected to decrease with increasing distance from the end of the aluminum plate due to reactions between the wall surfaces and the gas phase.

Figure 6 shows the lengths of radish sprouts 7 days after oxygen plasma irradiation as a function of l for the cases with and without the aluminum plate as well as for radish sprouts that had not been subjected to plasma irradiation (control). The sprout length obtained for $l = 25$ cm is nearly equal to that when the aluminum plate was not present. The sprout length decreases with increasing l. These results imply that oxygen-related species play an important role in the growth enhancement, whereas photons and ions do not. Possible oxygen species that cause the growth enhancement are O atoms in the ground state and metastable states and O_2 molecules in metastable states since O_2 gas does not enhance growth and the lifetimes of these species are expected to be longer than their characteristic diffusion times (i.e., below the order of milliseconds). Further study is necessary to precisely identify the species responsible for the growth enhancement.

The other possible growth stimulation mechanism is etching of seed husks by O_2 plasma irradiation, which may stimulate germination.[26,27] We observed seed surfaces by SEM and examined the chemical bonds on their husks by FTIR. Figure 7 shows SEM images of seed surfaces obtained after various plasma irradiation times. The surface morphology of seeds does not change; specifically, no cracks or holes are visible on the surface even after irradiation for 120 min. Figure 8 shows FTIR-ATR spectra of the husk surface. The spectrum of seeds with oxygen radical irradiation is identical to that of seeds that had not been irradiated. Furthermore, no oxides formed by oxygen radicals could be detected. These results demonstrate that the surfaces of the sprout seeds are not modified by O_2 plasma irradiation. Moreover, O_2 plasma irradiation does not affect seed germination. All these results indicate that stimulation of germination by etching of the seed husks does not cause growth enhancement.

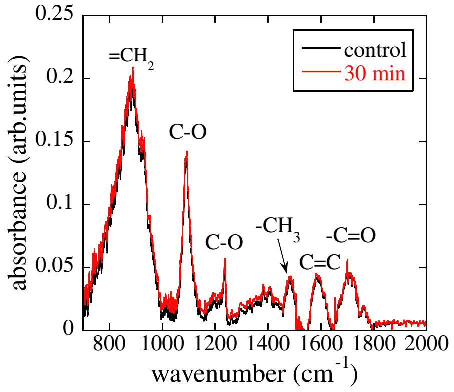

Fig. 8. (Color online) FTIR spectra of seed husks that have been subjected to 30 min O_2 plasma irradiation and that have not been irradiated.

We are currently performing experiments to investigate whether the growth enhancement mechanism involves a transcription factor or DNA.

4. Conclusions

We have investigated growth enhancement of radish sprouts induced by low pressure O_2 RF discharge plasma irradiation. We conclude the following:

1. O_2 plasma irradiation enhances the growth of radish sprouts but it does not affect seed germination.
2. Sprouts that had been irradiated by an O_2 plasma were 30–60% longer than those that had not been irradiated.
3. Oxygen radicals play an important role in enhancing growth, whereas photons and ions do not.
4. O_2 plasma irradiation does not significantly modify the surfaces of sprout seeds.
5. O_2 plasma irradiation does not affect seed germination.

Acknowledgment

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