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Plasma induced long-term growth enhancement of *Raphanus sativus* L. using combinatorial atmospheric air dielectric barrier discharge plasmas

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A B S T R A C T

Combinatorial analysis has been carried out to investigate the long-term effects of plasma irradiation of radish seeds on the subsequent sprout growth (*Raphanus sativus* L.) using atmospheric dielectric barrier discharge plasmas in air. The average seedling length was maximized with 180 s of plasma irradiation when the seed was 5 mm from the electrode edge and 3 mm below the electrode. With these parameters the average seedling length was 250% longer than that not irradiated after three days of cultivation. Observation of the seeds using an infrared (IR) camera and scanning electron microscopy revealed that the temperature rise and etching of the seeds during the plasma irradiation have little effect on growth enhancement. The interaction between radicals and seeds for a duration of 180 s leads to the growth enhancement of radish sprouts for 7 days.

1. Introduction

The recent global food crisis has motivated developing countries and the international community to revitalize global agriculture [1]. A possible solution to the global food crisis is to improve agricultural productivity. A novel means of achieving this is through the use of plasma [2–5].

Atmospheric pressure non-thermal plasmas have recently been employed in agricultural and medical applications because they cause little damage to biological materials [6–8]. Growth enhancement of mammalian and plant cells has been studied using atmospheric pressure discharge plasmas [9–14]. In such applications, controlling the dose and flux of radicals is important for clarifying growth enhancement mechanisms and optimizing the effects of the plasma. To gain an insight into such mechanisms, plasma irradiation needs to be carried out under various conditions. Radical generation and loss rates in air plasmas are sensitive to humidity, resulting in poor reproducibility in terms of dose and flux [15]. To overcome this poor reproducibility, we have applied a combinatorial method which realizes plasma irradiation under various conditions simultaneously using a spatially varying particle concentration. Such combinatorial methods are revolutionizing the approach to synthetic organic chemistry and other fields, and are greatly accelerating the rate of discovery of new molecular entities [16–25]. In low pressure plasma processes, large amounts of data can be obtained simultaneously by creating a spatial gradient in the particle density [26–30]. We expect similar acceleration of plasma based identifying processes and mechanisms in agricultural fields using the combinatorial approach.

In the present study we investigated the growth enhancement of *Raphanus sativus* L. (radish sprouts) using a combinatorial plasma irradiation method with an atmospheric dielectric barrier discharge (DBD) device. We also measured the concentrations of NO_x and O_3 produced by the device to elucidate their correlation to the growth enhancement of radish sprouts.

2. Experimental

Experiments were carried out with a scalable dielectric barrier discharge (DBD) device as shown in Fig. 1. The device consisted of

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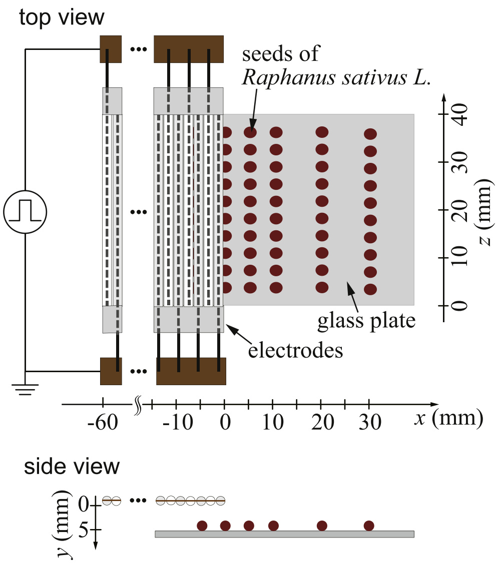

Fig. 1. Schematic of combinatorial plasma irradiation using a scalable DBD discharge device.

20 stainless steel rod electrodes 1 mm in diameter and 60 mm in length, covered with ceramic tubes 2 mm in outer diameter. They were arranged with a spacing of 0.2 mm. A DBD was generated in the air between the electrodes by supplying a 10 kHz AC high voltage (Logy Electric, LHV-09K). The discharge voltage and current were measured with a high-voltage probe (Tektronix, P6015A) and a Rogowski coil (URD, CTL-28-S90-05Z-1R1), respectively. The peak-to-peak discharge voltage and current were 9.2 kV and 0.2 A, respectively. The corresponding discharge power density was 1.49 W/cm^2, which was deduced from voltage/charge Lissajous plots. To obtain the spatial profiles of the radical density, we measured the concentrations of NO, NO_2, and O_3 using gas detector tubes (GASTEC, GV-100). The O_3 radical density was also measured by optical absorption spectroscopy using a deuterium lump (Hamamatsu Photonics, L11799).

For the combinatorial plasma irradiation, dry radish seeds were set in lines parallel with the z axes at $x = -5$, 0, 5, 10, 20, and 30 mm, with 10 seeds per line, as shown in Fig. 1. The irradiation was carried out with $y = 3$, 5, and 10 mm, where y is the height of the electrodes above the seeds. The experiments were carried out three times to confirm reproducibility. The temperature and relative humidity of the air were 24–26 °C and 57–65%, respectively. The irradiation duration was 180 s. We generated the discharges in still air so the seeds were not disturbed by an air flow. The species generated by the discharges were transported from the plasma to the seeds by free convection or diffusion. The surface temperature of the seeds was measured with an IR camera (NEC, TH7800N) to study the effects of the heat flux from the discharges on the growth of the sprouts. The surfaces of the seeds were examined using scanning electron microscopy (SEM; Hitachi S-3400N). Seeds with and without plasma irradiation were cultivated in a plant incubator at 22 °C and 60% relative humidity in the dark with pure water feed. The total length of the radish sprouts (i.e., from the root to the top of the stalk) was measured with an image analysis system.

3. Results and discussion

For combinatorial plasma irradiation, radical density should vary spatially in the treatment area. To confirm the spatial density distribution of the radicals generated with the scalable DBD device, we measured the x dependence of the NO_x and O_3 concentrations for the different values of y, as shown in Fig. 2(a) and (b). The O_3 concentration for $y = 3$ mm monotonically decreases from 180 ppm at $x = -5$ mm to 0 ppm at $x = 10$ mm. The NO_x concentration decreases from 320 ppm at $x = -5$ mm to 0 ppm at $x = 20$ mm. Both the O_3 and NO_x concentrations also decrease with increasing y. In atmospheric air discharges, the most common NO_x species are NO and NO_2. We measured the x dependence of the NO and NO_2 concentrations individually for $y = 3$ mm as shown in Fig. 2(c). The concentration of NO decreases from 300 ppm at $x = -5$ mm to 0 ppm at $x = 10$ mm, whereas that of NO_2 is 15 ppm between $x = -5$ mm and $x = 5$ mm but decreases to 0 by $x = 15$ mm.

O_3, NO and NO_2 are generated by the following major reactions in the discharge region [15,31–34].

$$e + O_2 \rightarrow 2O + e \tag{1}$$

$$O + O_2 + M \rightarrow O_3 + M \quad k = 3.4 \times 10^{-34}\ \mathrm{cm^6/s} \tag{2}$$

$$e + N_2 \rightarrow e + 2N \tag{3}$$

$$N + O_2 \rightarrow NO + O \quad k = 7.7 \times 10^{-17}\ \mathrm{cm^3/s} \tag{4}$$

$$N + O_3 \rightarrow NO + O_2 \quad k = 3.7 \times 10^{-13}\ \mathrm{cm^3/s} \tag{5}$$

$$O + NO_2 \rightarrow NO + O_2 \quad k = 9.7 \times 10^{-12}\ \mathrm{cm^3/s} \tag{6}$$

$$NO + O_3 \rightarrow NO_2 + O_2 \quad k = 2.1 \times 10^{-14}\ \mathrm{cm^3/s} \tag{7}$$

$$H_2O + e \rightarrow OH + H + e \tag{8}$$

$$H + O_2 + M \rightarrow HO_2 + M \quad k = 1.8 \times 10^{-32}\ \mathrm{cm^6/s} \tag{9}$$

$$NO + HO_2 \rightarrow NO_2 + OH \quad k = 7.8 \times 10^{-12}\ \mathrm{cm^6/s} \tag{10}$$

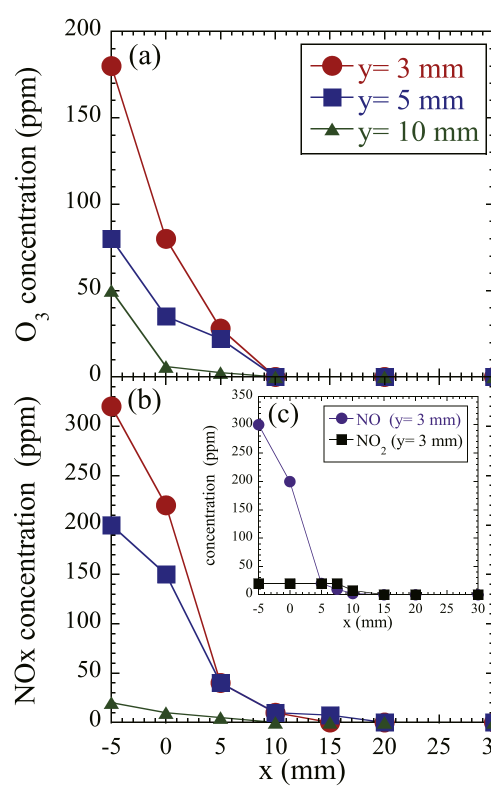

Fig. 2. Spatial profiles of concentrations of (a) O_3, (b) NO_x and (c) NO and NO_2 for $y = 3$ mm.