Growth enhancement effects of radish sprouts: atmospheric pressure plasma irradiation vs. heat shock

T Sarinont¹, T Amano², S Kitazaki³, K Koga¹, G Uchida⁴, M Shiratani¹ and N Hayashi³

¹Graduate School of Information Science and Electrical Engineering, Kyushu University 744 Motooka, Nishi-ku, Fukuoka, 819-0395, Japan
²Department of Electrical Engineering, School of Engineering, Kyushu University 744 Motooka, Nishi-ku, Fukuoka, 819-0395, Japan
³Interdisciplinary Graduate School of Engineering Science, Kyushu University 6-1 Kasuga-kouen, Fukuoka, 816-8580, Japan
⁴Joining and Welding Research Institute, Osaka University, 11-1 Mihogaoka, Ibaraki, Osaka, 567-0047, Japan

E-mail: t.sarinont@plasma.ed.kyushu-u.ac.jp

Abstract. We compare growth enhancement effects due to atmospheric air dielectric barrier discharge plasma irradiation and heat shock to seeds of radish sprouts (Raphanus sativus L.). Interactions between radicals and seeds in a short duration of 3 min. lead to the growth enhancement of radish sprouts in a long term of 7 days and the maximum average length is 3.7 times as long as that of control. The growth enhancement effects become gradually weak with time, and hence the ratio of the average length for plasma irradiation to that for control decreases from 3.7 for the first day to 1.3 for 7 day. The average length for heat shock of 60°C for 10 min. and 100°C for 3 min. is longer than that for control, and the maximum average length is 1.3 times as long as that of control. Heat shock has little contribution to the growth enhancement due to plasma irradiation, because the maximum temperature due to plasma irradiation is less than 60°C.

1. Introduction

The demand for food will continue to increase towards 2050 as a result of population growth by an additional 2.7 billion people [1]. Increases in food production per hectare of land have not kept pace with increases in population, leading to the global food crisis. Especially, the current world food crisis is the result of the combined effects of competition for cropland from the growth in biofuels, low cereal stocks, high oil prices, speculation in food markets and extreme weather events [1]. One possible solution to the global food crisis is to improve agricultural productivity by some means. Most improvement methods of agricultural productivity are irradiations of γ-ray, ⁶⁰Co-ray, laser, electric and magnetic field. Such irradiations often induce damage of cells of plant seeds [2-4]. Atmospheric pressure plasmas become prevalent in various applications, ranging from material processing to biomedicine [5-20]. Atmospheric pressure non-thermal plasmas have recently been employed to agricultural crops and the accelerate seed germination in order to improve agricultural productivity [5]. For interactions with living tissues, the choice of an adequate plasma source is crucial. It was operated under atmospheric pressure, electrically and chemically safe, and may not cause any thermal damage.
to the living object. Recently, we have found that atmospheric pressure dielectric barrier discharge (DBD) plasma irradiation to seeds of plants enhance their growth [16-20]. The growth promotion is attributed not to an increase in size of each cell but to an increase in the number of cells. Plasma irradiation may raise the temperature of seeds. In many species, plants germination and growth are significantly affected by the temperature treatment level [19-20]. For instance, short exposure of seeds to high temperatures generally stimulates germination, whereas prolonged exposure reduces seed germination [21-26]. Here, we focus on the effects of temperature rise due to plasma irradiation to seeds of radish sprouts (Raphanus sativus L.) on the plant growth.

2. Experimental
Experiments were carried out using a atmospheric pressure scalable DBD device described elsewhere [16-20]. The device consisted of 20 electrodes of a stainless rod of 1 mm in outer diameter and 60 mm in length covered with a ceramic tube of 2 mm in outer diameter as shown in Figure 1. The electrodes were arranged parallel at spacing of 0.2 mm. DBD plasma was generated in air between the electrodes by supplying 10 kHz AC high voltage (Logy Electric, LHV-09K). The temperature and humidity of air were 24-26°C and 57-61%, respectively. 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/cm², which was deduced from voltage/charge Lissajous plots. There was little air flow around the DBD device, and therefore, spatial profiles of radical densities were governed by diffusion and gas phase reactions.

10 seeds of radish sprouts (Raphanus sativus L.) were arranged horizontally at intervals of 5 mm at 3 mm below the electrodes DBD plasma was irradiated to the seeds for 3 min. Seeds with and without plasma irradiation were cultivated for 7 days in an incubator at 22°C and 60% relative humidity in the dark with pure water feed. Their total length from primary root to stark was measured with an image analysis system and statistical significance of the total length was evaluated every day.

We also examined effects of heat shock. To apply heat shock, 10 seeds in a quartz petri dish were put on hot plate. The hot plate temperature was set at 60°C or 100°C for 3 min. or 10 min. Then the seeds were cooled down at room temperature or -20°C for 10 min. Seeds with and without heat shock
were cultivated for 3 days in an incubator at 22℃ and 60% relative humidity in the dark with pure water feed. Their total length was measured every day. Time evolution of the surface temperature of seeds was measured with an IR-camera (NEC, TH7800N).

3. Results and discussion

First, we examined effects atmospheric pressure DBD plasma irradiation to seeds on growth of plants. Figure 2(a) shows growth curves for radish sprouts with and without plasma irradiation. The symbols and bars in figure 2(a) show the average length and standard error, respectively. The length with plasma irradiation is longer than that without plasma irradiation for 7 days. Interactions between seeds and radicals produced by the plasmas in a short duration of 3 min. lead to the growth enhancement of radish sprouts in a long term of 7 days. Figure 2(b) shows time evolution of a ratio \( \frac{l_{\text{plasma}}}{l_{\text{control}}} \) of the average length for plasma irradiation to that for control together with a difference \( l_{\text{plasma}} - l_{\text{control}} \) between the length for plasma irradiation and that for control. The difference increases with time during 7 days, namely, plasma irradiation promotes plant growth for 7 days. The ratio decreases from

![Figure 2.](image)

Figure 2. (a) Growth curve of plants and (b) time evolution of a ratio between the length for plasma irradiation and that for control together with a difference between the length for plasma irradiation and that for control.

![Figure 3.](image)

Figure 3. Time evolution of surface temperature of seeds on heat treatments for (a) 3min. and (b) 10 min.
3.7 at the first day to 1.3 at 7 day. Therefore, the response of plants to the plasma irradiation becomes gradually weak with time.

Plasma irradiation to seeds raises the temperature of seeds up to 30°C. Such heat shock due to plasma irradiation may contribute to the growth enhancement in figure 2. To clarify effects of heat shock to the seeds, dry heat treatments to seeds in a quartz petri dish were carried out with a hot plate. 8 kinds of heat treatments were applied to seeds: (1) They are heated on hot plate of 60°C for 3 min. then they are cooled down to room temperature, (2) They are heated on hot plate of 100°C for 3 min. then they are cooled down to room temperature, (3) They are heated on hot plate of 60°C for 3 min. then they are cooled down to -20°C, (4) They are heated on hot plate of 100°C for 3 min. then they are cooled down to -20°C, (5) They are heated on hot plate of 60°C for 10 min. then they are cooled down to room temperature, (6) They are heated on hot plate of 100°C for 10 min. then they are cooled down to room temperature, (7) They are heated on hot plate of 60°C for 10 min. then they are cooled down to -20°C, (8) They are heated on hot plate of 100°C for 10 min. then they are cooled down to -20°C.

Figures 3(a) and 5(b) show time evolution of the seed surface temperature. The seed surface temperature gradually increases with time. For the heat treatment of 3 min., the highest seed surface

![Figure 4. Growth curve of plants for control and .(1) heated on 60°C plate for 3 min. then cooled down to room temperature, (2) heated on 100°C plate for 3 min. then cooled down to room temperature, (3) heated on 60°C plate for 3 min. then cooled down to -20°C, (4) heated on 100°C plate for 3 min. then cooled down to -20°C, (5) heated on 60°C plate for 10 min. then cooled down to room temperature, (6) heated on 100°C plate for 10 min. then cooled down to room temperature, (7) heated on 60°C plate for 10 min. then cooled down to -20°C, (8) heated on 100°C plate for 10 min. then cooled down to -20°C.](image1)

![Figure 5. Average length of Raphanus sativus L. after 3 days cultivation for control and .(1) heated on 60°C plate for 3 min. then cooled down to room temperature, (2) heated on 100°C plate for 3 min. then cooled down to room temperature, (3) heated on 60°C plate for 3 min. then cooled down to -20°C, (4) heated on 100°C plate for 3 min. then cooled down to -20°C, (5) heated on 60°C plate for 10 min. then cooled down to room temperature, (6) heated on 100°C plate for 10 min. then cooled down to room temperature, (7) heated on 60°C plate for 10 min. then cooled down to -20°C, (8) heated on 100°C plate for 10 min. then cooled down to -20°C.](image2)
temperature is 35°C for the hot plate temperature of 60°C and 48°C for the hot plate temperature of 100°C. For the heat treatment of 10 min., the highest seed surface temperature is 35°C for the hot plate temperature of 60°C and 55°C for the hot plate temperature of 100°C.

Figure 4 shows time evolution of the average length of *Raphanus sativus* L. and figure 5 shows the average length after 3 days cultivation. Temperature had a significant influence on the germination of seeds of plant since first day after cultivation as shown on Figure 4. The heat shock treatments of (1)-(5), and (7) leads to growth enhancement and the length ratio of heat treatment plants to control after 3 days cultivation ranges from 1.2 to 1.4, which are much below the ratio of 2 for plasma irradiation. The heat treatments of (6) and (8) suppress significantly the growth. Both the temperature and the duration of treatments have influence on germination and growth of plants. The low temperature heat treatments tend to enhance germination, whereas high temperature short heat treatments enhance growth of plants. However, high temperature long heat treatments suppress germination, which are similar to the results in [25].

Based on the results in figures 2-5, the temperature rise of seeds due to plasma irradiation has little effects on the plant growth, because the maximum surface temperature of seed during plasma irradiation is 30 °C. Moreover our previous results show that irradiation of photons and ions to seeds has little effects on plant growth. Therefore, interactions between radicals such as OH, H₂O₂, O and O⁻ and seeds may bring about growth enhancement of plants in a long term of 7 days. One possible mechanism is that plasma irradiation leads to secrete some hormones in seeds, intracellular chemical signals and messengers induce the growth enhancement.

4. Conclusions
We applied plasma irradiation and heat shock to seeds of radish sprouts to study their effects of growth of radish sprouts. We obtained the following conclusions:
1) Plasma irradiation for 3 min. enhances plant growth in a long term of 7 days.
2) The response of plants to the plasma irradiation becomes gradually weak with time, and the ratio of plant length with plasma irradiation to control decreases from 3.7 at the first day to 1.3 at 7 day.
3) Temperature rise during plasma irradiation has little effect on the growth enhancement.

The remaining issues of clarification of the growth enhancement mechanisms are identification of plasma generated species responsible for the growth enhancement, and stimulated signal pathways in the plants for the growth enhancement.

Acknowledgement
This work was supported by JSPS KAKENHI grant number 2434014.

References
[1] Nellemann C, MacDevette M, Manders T, Eickhout B, Svihus B, Prins A G and Kaltenborn B P 2009 *The environmental food crisis – The environment’s role in averting future food crises* ISBN: 978-82-7701-054-0
[2] Nastuta A, Topala I, Grigoras C, Pohoata V and Popa G 2011 *Jpn. J. Appl. Phys.* 44 105204
[3] Pimentel D, Hurd L E, Bellotti A C, Forster M J, Oka I N, Shores O D and Whitman R J 1973 *Science* 182 4111
[4] Victor J, Thannickal V and Fanberg B 2000 *Am. J. Physiol.* 279 L1005
[5] Lee J, Shon C H, Kim Y S, Kim S, Kim G C and Kong M G 2009 *New J. Phys.* 11 115026
[6] Fridman A, Chirokov A and Gutsol A 2005 *Jpn. J. Appl. Phys.* 38 R1
[7] Chirokov A, Gutsol A and Fridman A 2005 *Pure Appl. Chem.* 77 487
[8] Ito M, Ohta T and Hori M 2012 *J. Korean Phys. Soc.* 60 6
[9] Zhou Z 2011 *Agr. Sci.* 2 23
[10] Kong M G, Kroesen G, Nosenko T, Shimizu T, Van Dijk J and Zimmermann J 2009 *New J. Phys.* 11 115012
[11] Laroussi M and Leipold F 2004 *Int. J. Mass Spectrom.* 233 81
[12] Stoffels E, Keift I E and Sladek R E J 2003 Jpn. J. Appl. Phys. 36 2908
[13] Dobrynin D, Fridman G, Friedman G and Fridman A 2009 New J. Phys. 11 115020
[14] Hayashi N, Tsutsui S, Tomari T and Guan W 2008 IEEE Trans. on Plasma Science 36 1302
[15] Hayashi N and Yagyu Y 2008 Trans. of the Material Research Society of Japan 33 791
[16] Kitazaki S, Yamashita D, Matsuzaki H, Uchida G, Koga K and Shiratani M 2010 Proc. IEEE TENCON 1960
[17] Kitazaki S, Koga K, Shiratani M and Hayashi N 2012 MRS Proc. 1469
[18] Kitazaki S, Koga K, Shiratani M and Hayashi N 2012 Jpn. J. Appl. Phys. 51 11PJ02
[19] Kitazaki S, Sarinot T, Koga K, Shiratani M and Hayashi N 2014 Current Appl. Phys. in press
[20] Sarinont T, Kazunori Koga, Satoshi Kitazaki, Giichirou Uchida, Nobuya Hayashi and Masaharu Shiratani 2014 JPS Conf. Proceedings in press
[21] Wehmeyer N, Hernandez L D Finkelstein R R and Vierling E 1996 Plant Physiol. 112 747
[22] Gashaw M and Michelson A 2002 Plant Ecol. 159 83
[23] Schoffl F, Prandl R and Reindl A 1998 Plant Physiol. 117 1135
[24] Gashaw M and Michelsen A 2001 Plant Ecol. 159 83
[25] Thomas B P, Morris E C and Auld D T 2003 Austral Ecol. 28 674
[26] Boehm K A, Saunders A, Werner J and Lis T J 2003 Mol. Cell. Biol. 23 7628