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Growth Promotion of Komatsuna (*Brassica rapa* var. *perviridis*) by Ozonated Water Supplied Intermittently to Underground Roots

Satoru Iizuka[a,*] & Hideyuki Saito[b]

[a]Department of Electrical Engineering, Graduate School of Engineering, Tohoku University, 6-6-05 Aza Aoba, Aramaki, Aoba, Sendai, Miyagi 980-8579, Japan; [b]School of Food, Agricultural, and Environmental Sciences, Miyagi University, 2-2-1 Hatadate, Taihaku, Sendai, Miyagi 982-0215, Japan

*Address all correspondence to: Satoru Iizuka, Department of Electrical Engineering, Graduate School of Engineering, Tohoku University, 6-6-05 Aza Aoba, Aramaki, Aoba, Sendai, Miyagi 980-8579, Japan; Tel.: 81-22-795-7113; Fax: 81-22-263-9374, E-mail: iizuka@ecei.tohoku.ac.jp

ABSTRACT: An intermittent supply of ozonated water to underground roots revealed a promotive effect on the growth of komatsuna (*Brassica rapa* var. *perviridis*). Although no clear difference in germination time and early growth was seen, a definite difference appeared ~27 d after germination. A remarkable difference was observed in growth rate after 49 d, whereas growth rate in the control was nearly saturated. Plant weight after 49 d increased by 2–3 times, compared to that of the control. An intermittent supply of a suitable amount of ozonated water to the underground soil increased plant growth promotion.

KEY WORDS: ozonated water, corona discharge, plant growth enhancement, plant growth promotion

I. INTRODUCTION

Ozone, one of the strongest oxidizing and bleaching agents, is beneficial in that it can decay without leaving residues that could harm the environment. Because ozone is produced easily by air discharge, it is increasingly used for many kinds of oxidizing processes including as sterilization and disinfection.[1] In nature, ozone is produced by ultraviolet irradiation, and ground levels of ozone cause more damage to plants than all other air pollutants combined. When entering plants through leaf stomata during normal gas exchange, ozone induces fatal damage, such as chlorosis and necrosis.[2–4]

The effect of ozone on plants depends on the concentration and duration of the exposure.[5] Low-levels exposure of ozone result in a decline in photosynthesis, growth inhibition, and premature senescence, normally without visible damage to plant tissues.[6] In contrast, high ozone doses within a short time frame leads to cell death and visible lesion formation in sensitive plants.[7,8] It should be noted that plant response to ozone with elevated CO_2 has a different effect on photosynthesis and growth.[9–12] Soybeans grown at different combinations of CO_2 and ozone, for example, showed enhanced rates of photosynthesis.[13,14]

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Acute ozone exposure induces massive changes in gene expression, enzyme activities, and metabolic profiles, even when no tissue damage is detected.[15–18] Changes in protein activities and gene expression can occur very quickly, sometimes within minutes after the onset of exposure. A common element in ozone and pathogen responses is active production of reactive oxygen species (ROS) in the apoplast, the extracellular space. Thus, ozone can also be used as a noninvasive tool to mimic signaling pathways triggered by active apoplastic ROS formation, and results obtained in experiments that use ozone directly relate to understanding the role of apoplastic ROS signaling in general. The signaling pathways activated by ozone are integrated into a complex regulatory system involving ROS, plant stress hormones, and second messengers such as calcium.[5]

It has also been shown that a plant response induced by brief exposure of ozone leads within minutes to activation of mitogen-activated protein kinase (MAPK).[19] This activation process is calcium dependent and can be associated with induction of ROS accumulation. Rapid MAPK activation indicates that ROS themselves may be responsible for triggering signaling through MAPK cascades. Because ozone immediately creates ROS in plant tissues, exposure to ozone may also lead to rapid MAPK activation.[20] It should be noted that MAPK pathways regulate diverse processes, ranging from proliferation and differentiation to apoptosis. Activated by an enormous array of stimuli, MAPK pathways phosphorylate numerous proteins, including transcription factors, cytoskeletal proteins, kinases, and other enzymes, and greatly influence gene expression, metabolism, cell division, cell morphology, and cell survival.[21] In relation to proliferation and cell survival, the surviving cells have the ability to repair some oxidative damage,[22,23] strongly suggesting that this repair is an essential step for resumption of growth. The nature of the repair mechanisms involved is unknown. However, reduction of oxidized sulfhydryl compounds in autotrophic cells is largely dependent on photosynthetic production of nicotinamide-adenine dinucleotide phosphate (NADPH).[24] Thus, studies on photosynthetic activity following exposure to ozone would undoubtedly provide useful information in elucidating the nature of the repair mechanisms involved.[25–27]

The object of our experiments is to clarify the effect of ozone on plant growth. We directly supplied ozonated water to the underground soil for plant cultivation.

II. EXPERIMENTAL APPARATUS AND METHOD

For plant growth, the absorption of nutrients from roots as well as the photosynthetic reaction in leaves are quite important. We focused on the activation of underground roots of plants by feeding ozonated water to the underground soil.[28] We cultivated komatsuna (*Brassica rapa* var. *perviridis*), Japanese mustard spinach, a leaf vegetable that may be eaten at any stage of its growth. Experiments were performed in a greenhouse in Tsubonuma Farm, Miyagi University, Japan. Ozonated water was generated using an ozonated water generator (Inpal Co. Ltd., Japan), in which ozone was produced efficiently by an atmospheric corona discharge in a narrow annular gap between an external grounded cylindrical electrode and inner coaxial rotating electrode, with a rotation speed of 1380 turns/min.[29]

We used eight plastic plant cultivation (planter) boxes that were 47.7 cm long, 32.6 cm wide, and 7.6 cm high, with several drain holes at the bottom. The ozonated water of 1 ppm at a flow rate of 0.6 L/min was delivered intermittently to the eight planter boxes by switching the magnetic valves on and off with the use of an electric timer (Autorain time switch FV811, SUNAO Electric Co. Ltd., Japan). The main timer switch was turned on and off automatically twice a day within 10 min between 9:00 am and 11:00 am (except on rainy and cloudy days) during cultivation from sowing to harvesting. Feeding treatment times of ozonated water to each planter box were fixed to 5, 10, 15, 30, 60, 78, and 90 s for planter boxes 1–8, respectively. For the control, tap water with the same flow rate of 0.6 L/min was fed for 10 s. Details are described elsewhere.[28]

The planter box layout is shown in Fig. 1. Cultivation soil (Sukoyaka, Yamagata Celltop Co. Ltd., Japan) was packed in the box at a depth of 5 cm. The box was divided into two areas by a partition plastic plate, inserted vertically 3 cm into the soil. One area was for cultivation and the other for ozonated water feeding, where a polyethylene terephthalate (PET) bottle (2 L volume) was placed. Because the PET bottle was buried into the soil at 2 cm in depth, the ozonated water was able to leak into the underground soil directly through five holes of 1 cm in diameter, opened in the vertical lower side of the PET bottle. The processed water penetrated into the cultivation area through a narrow channel below the partition panel that was placed between the two areas. Here, 100 seeds of komatsuna (Cultivar Maruha, Watanabe Seed Co. Ltd., Japan) were sowed along the y direction defined in Fig. 1, with five rows in the x direction perpendicular to the y direction. The raw positions were spaced at x = 3, 6, 9, 12, and 15 cm. After performing

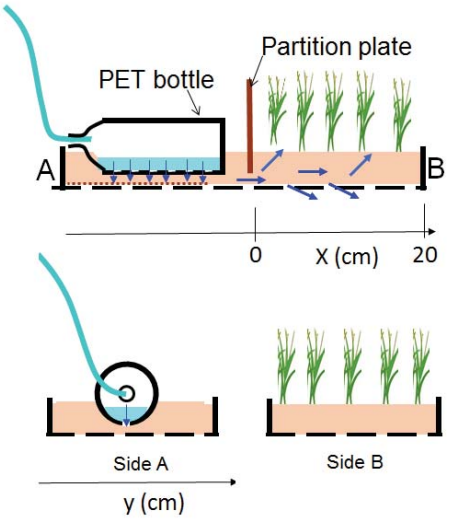

FIG. 1: Layout of the planter box. Ozonated water of 1 ppm with a flow rate of 0.6 L/min is supplied to the underground soil, and the processed water penetrates into cultivation area ($x \geq 0$) through the narrow channel beneath a partition plate inserted into the soil at $x = 0$.

appropriate thinning, 25 samples of komatsuna (five samples in the x direction by five samples in the y direction) were harvested on the 49th d after sowing. To adjust the total amount of water feeding to the planters, tap water was sprinkled at 4:00 pm.

III. EXPERIMENTAL RESULTS

The distance of 3 cm in the middle cultivation region was too narrow for the plant to grow without a mutual shielding effect. For this reason, only plants at the both ends of the rows, i.e., at x = 3 and 15 cm, were taken into account. Histograms of the plant height of five komatsumas, harvested at x = 15 cm on the 49th d after sowing, are shown in Fig. 2 in eight cases. In the case of the control, the average height was 32.0 cm and standard deviation was 4.7 cm. The average height increased with treatment time and reached

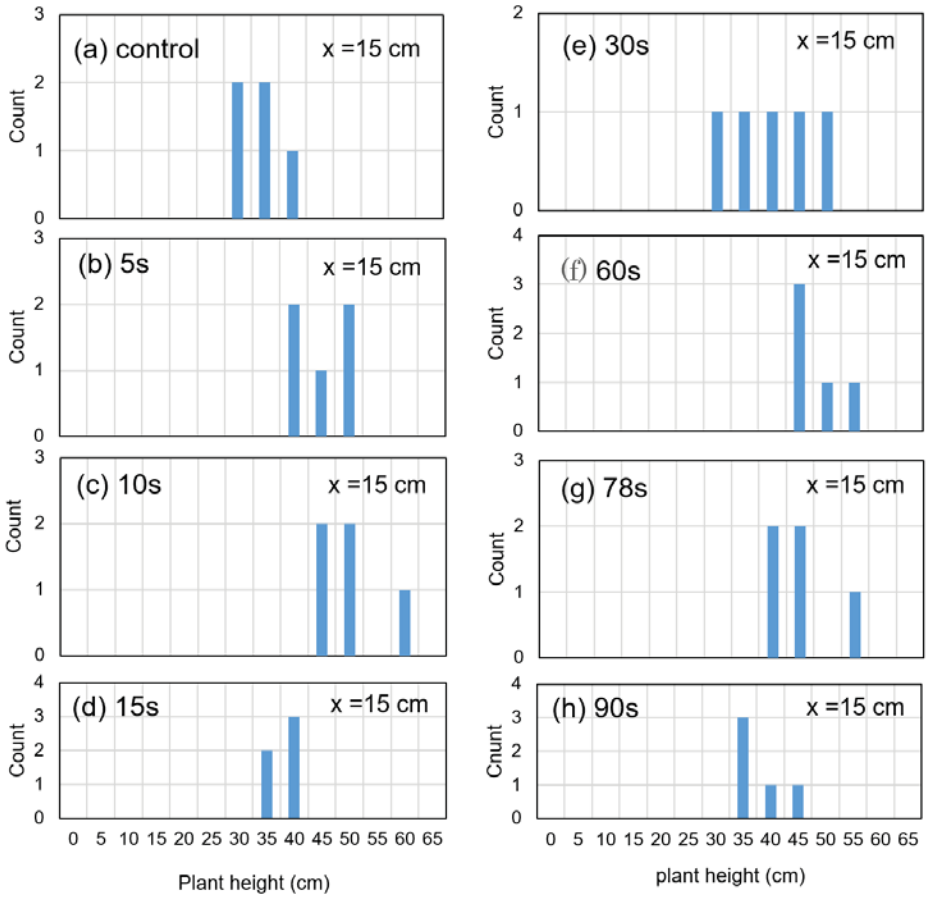

FIG. 2: Histograms of the plant height of five Komatsunas sampled at x = 15 cm on the 49th d after sowing in the cases of (a) control and treatment times of (b) 5 s, (c) 10 s, (d) 15 s, (e) 30 s, (f) 60 s, (g) 78 s, and (h) 90 s.

the first maximum of 47.4 cm in the case of the 10-s treatment and then decreased to the minimum in the case of the 15-s treatment. With increased treatment time, average height increased again and then reached the second maximum of 43.8 cm in the case of the 78-s treatment. However, additional treatment time resulted in a decrease in the average value again.

To compare these differences, see the images of the average sizes of komatsunas sampled at x = 15 cm in the cases of 10- and 60-s treatments in Fig. 3(a) and (b), respectively, with the image of the control sampled at x = 15 cm. Clearly, not only did the plant height increase but so did the number of leaves, number of stems, and width and length of leaves in the cases of 10- and 60-s treatments, compared to those of the control.

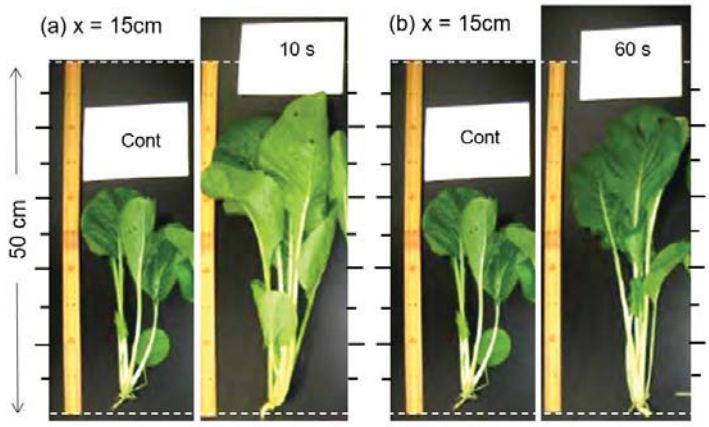

FIG. 3: Images of the average sizes of control and treatment times of (a) 10 s and (b) 60 s. All samples are harvested at x = 15 cm. A 50-cm-long ruler is placed aside, with scales every 5 cm. Cont, Control.

Variation in the average plant height sampled at x = 15 cm is plotted in Fig. 4, as a function of treatment time. Two peaks appear (A and B). Peak A was rather sharp and narrow, compared to the second broad peak of B. It was also remarkable that a minimum appeared between these peaks in the case of the 15-s treatment. This suggests that two different conditions exist for promoting growth in plants. The first appears within a very weak concentration of ozonated water. On the other hand, much more ozonated water is required for the appearance of the second peak B.

To investigate the growing process, temporal growth curves of the average plant height of five komatsunas sampled at x = 15 cm are plotted in Fig. 5 as a function of the number of days after sowing, with treatment time as a parameter. It can be clearly seen that both growth curves, at peak A in the case of the 10- treatment and peak B in the case of the 60-s treatment, increased almost in proportion to the number of days after sowing. On the other hand, the growth rate of the control decreased at ~27 d and then became

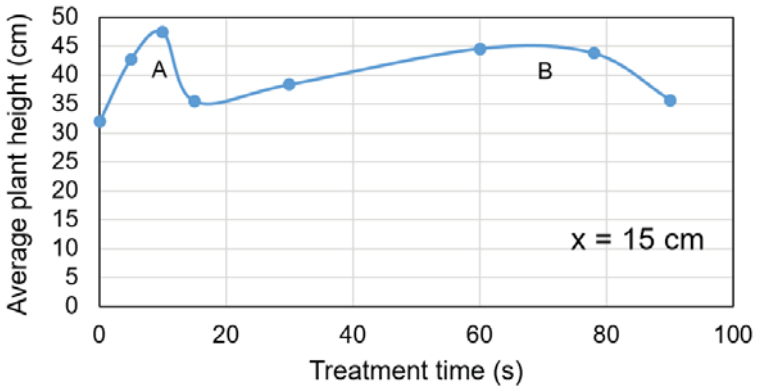

FIG. 4: Average plant height of five komatsunas sampled at x = 15 cm on the 49th d after sowing, as a function of treatment time. Two peaks (A and B) appear in the cases of 10- and 60-s treatments, respectively.

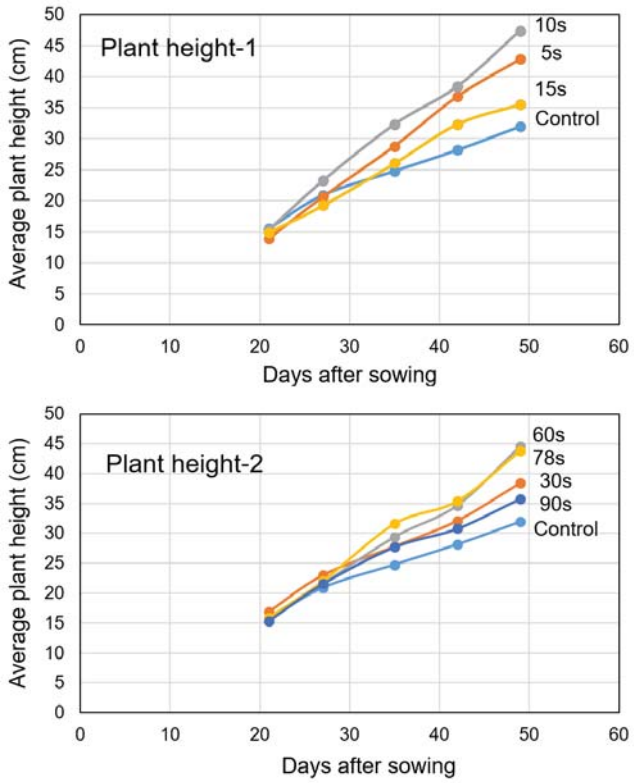

FIG. 5: Growth curves of the average plant height of five komatsunas sampled at x = 15 cm as a function of the amount of days after sowing, with treatment time as a parameter.

weak and almost saturated. It should be also noted that no clear difference was observed in germination time or in early growth until 27 d. A definite difference appeared at ~27 d after germination. Then, a remarkable difference was seen even after 49 d, although the growth rate in the case of control was nearly saturated.

Variations in the average weight as a function of treatment time with sampling position x as a parameter are shown in Fig. 6. Two peaks, A and B, occur in the curve at x = 15 cm, similar to the result in Fig. 4. Peaks A and B in Figs. 4 and 6 result from the same fact; that is, that the growth of the plants in the cases of 10- and 60- to 78-s treatments are promoted, compared to the control. Two peaks also appear at C and D in the curve at x = 3 cm, which may correspond to peaks A and B at x = 15 cm, respectively. The difference that treatment time becomes the maxima at x = 3 and 15 cm would result from an effect of three-dimensional diffusion of the supplied water, as discussed in the following section.

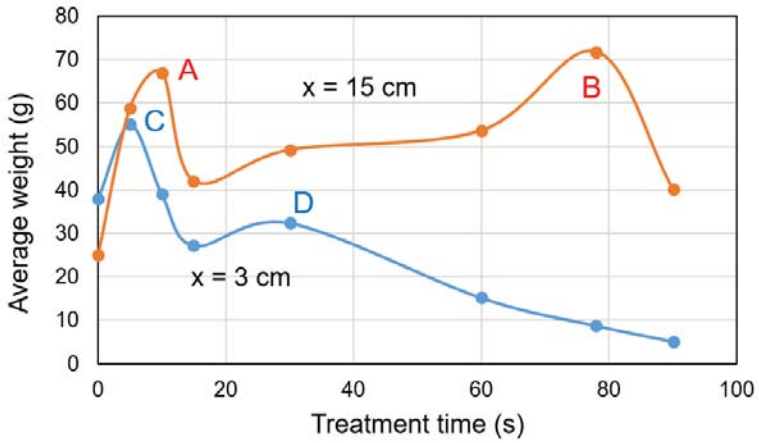

FIG. 6: Average plant weight of five komatsunas sampled at x = 3 and 15 cm on the 49th d after sowing, as a function of treatment time. Peaks A and B appear at x = 15 cm and peaks C and D at x = 3 cm.

IV. DISCUSSION

The amount of processed water arriving at x = 15 cm diminished spatially and temporally because of an absorption into the soil during penetration, resulting in the net amount of processed water arriving at x = 15 cm to be less, compared to that at x = 3 cm. Peaks A and B observed at x = 15 cm in Figs. 4 and 6 correspond to peaks C and D observed at x = 3 cm, respectively. The reduction factor of the processed water arriving at x = 15 cm can be estimated by adjusting both curves in such a way that the horizontal axis of x = 15 cm diminishes until peaks A and B agree with peaks C and D, respectively, as shown in Fig. 7. Here, the horizontal scale of the curve at x = 15 cm was reduced by a factor of

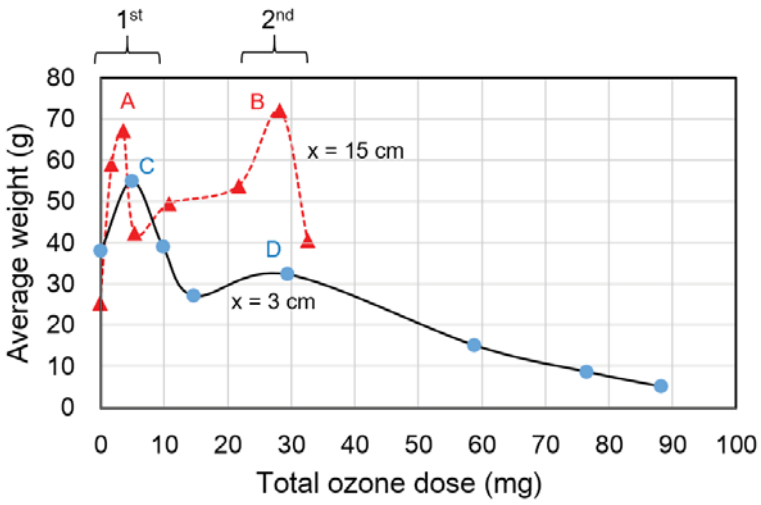

FIG. 7: Average plant height of five komatsunas sampled at x = 3 and 15 cm on the 49th d after sowing, as a function of the total ozone dose supplied to the underground soil in the feeding area ($x \leq 0$). Peaks A–D are the same described in Fig. 6.

2.7; that is, the processed water arriving at x = 15 cm was reduced by a factor of 2.7. As described above, the reducing factor was experimentally evaluated and depends on the ozone reaction with the soil, absorption of water, and soil denseness. Here, we adopted a soft cultivation soil, Sukoyaka, the weight of which was 16 kg for a volume of 40 L. This soil performs well in terms of water repellency and drainage capacity. Thus, the processed water can spread out three dimensionally, both to the sides and to the bottom of the planter boxes in the downstream region.

The total ozone dose supplied to the underground soil in the feeding area ($x \leq 0$) was calculated from the ozonated water concentration of 1 ppm multiplied by feeding treatment time, taking into account the flow rate of 0.6 L/min. Peaks C and D correspond to the total ozone dose of 5 and 30 mg, respectively, that was supplied to the soil in the feeding area ($x \leq 0$). The amount of ozone dose to the soil ($x \leq 0$) was on the order of milligrams, which was much smaller than the total mass of the soil at ~3.1 kg in the planter boxes.

Ozonated water supplied to the underground soil would more or less react with the soil before arriving at the plant roots. Therefore, in our case, three possibilities were regarded as an ozone effect. The first is the direct interaction between the root cells and ozone, in which cell proliferation and cell survival would occur by a defense mechanism.[19–23] The second is an indirect interaction via the reactive species produced by ozone in the soil. In this case, products such as nitrogen oxide might act as nutrients that would induce plant growth. The third is the combined effect of the first and second possibilities. To confirm such oxidization, we measured the pH of the soil in the cultivation area. We did not observe a definite change in pH, but nitrate nitrogen (NO_3-N) increased

in the ozonated-water–treated soil. The processed water arriving at the plant roots might contain such nitrogen oxides. However, the appearance of double peaks in plant growth promotion (A and B) could not be simply explained by the chemical change of the soil to nutrients. Severe suppression of plant growth observed at x = 3 cm in the case of treatment >60 s might be due to a negative effect of ozone.

In our experiment, ozonated water was intermittently supplied to the underground soil in the feeding area ($x \leq 0$) in a very short time twice daily, on everyday except rainy and cloudy days, although the ozone concentration in the water was rather strong at 1 ppm. Our results showed that such an intermittent supply rate of ozone is very effective for plant growth promotion.

V. CONCLUSION

Ozonated water produced by an atmospheric corona discharge was supplied intermittently to the underground soil ($x \leq 0$) for cultivation of komatsuna (*Brassica rapa* var. *perviridis*) by changing the feeding treatment time twice daily, everyday, for 49 d, except on rainy and cloudy days. Two levels of ozone dose resulted in plant growth promotion. These correspond to the total ozone doses of 5 and 30 mg, respectively, to the soil in the feeding area ($x \leq 0$). The average weights of komatsunas at x = 15 cm in the cases of 10- and 78-s treatment were 67.0 g and 71.8 g, respectively, whereas the average weight of the control at x = 15 cm was 25.0 g. Therefore, the average weight increased by 2.7 and 2.9 times in the cases of 10- and 78-s treatment, respectively, in comparison with the control. Suitable amounts of ozone supplied intermittently to the underground soil were found to promote plant growth.

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