Europ.J.Hort.Sci., **75** (5). S. 198–203, 2010, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

Promotion of Lettuce Growth by Application of Microbubbles in Nutrient Solution Using Different Rates of Electrical Conductivity and under Periodic Intermittent Generation in a Deep Flow Technique Culture System

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Summary

To investigate the effect of electrical conductivity (EC) in microbubbles-generated nutrient solution and periodic intermittent microbubble generation on lettuce growth, lettuce seedlings were cultivated at three different levels of EC (0.5, 1.0, or 2.0 dS m^{-1}) with microbubbles or at 1.0 dS m^{-1} with macrobubbles and under three levels of intermittent microbubbles generation (15, 30, and 60 min h^{-1}) in hydroponic culture for 2 weeks. Leaf fresh weight (LFW) of lettuce treated with 0.5 and 1.0 dS m^{-1} EC microbubbles was 1.54 and 1.43 times greater than those treated with macrobubbles. Although LFW of lettuce grown in 2.0 dS m^{-1} was significantly less than 0.5 and 1.0 dS m^{-1} conditions, there was no difference in leaf dry weight (LDW) among the three ECs microbubble conditions. There were no significant differences in the fresh and dry weights of lettuce treated with periodic intermittent generation of microbubbles for 15, 30 and 60 min h^{-1} (Cont.) regardless of significant difference between microbubble and macrobubble treatments. These results indicate that the microbubble generation in nutrient solutions can promote lettuce growth and intermittent microbubble generation for 30 min h^{-1} or less can be adapted effectively in hydroponic culture systems.

Key words. agricultural application – *Lactuca sativa* – macrobubbles – pH

Introduction

Microbubbles are tiny gas bubbles with mean diameters of less than 50 μm (Takahashi et al. 2003; Takahashi 2005). Microbubbles have been applied in many fields, including foam fractionation, food processing, purification of polluted water, and marine culture of oysters (Komada et al. 2000; Takahashi 2005; Ohnari and Tsunami 2006). When formed in water solutions, microbubbles decrease in size and ultimately disappear. Air microbubbles generate free radicals as they collapse and this has been suggested to increase the ion concentration around the gas-water interface (Takahashi et al. 2007). Because the surface of microbubbles is negatively charged (ζ potential) (Takahashi 2005; Ohnari and Tsunami 2006), they do not attract each other but rather increase in surface area and attract positively charged materials or ions.

Park and Kurata (2009) reported that the fresh and dry weights of microbubble-treated lettuce were significantly greater over a 2-week period than those of macrobubble-treated lettuce. Additionally, they found that the properties of the microbubble-generated nutrient solution, i.e. pH, electrical conductivity (EC), oxidation reduction potential, and dissolved oxygen (DO) content, were not considerably different from those of the macrobubble-generated nutrient solution. Because the size distribution and number of generated microbubbles depends on water quality and conditions (Takahashi et al. 2003), the properties of microbubbles generated in a particular solution may vary depending on the solute concentration. However, little information is available on the effects of ion concentrations in microbubble-generated nutrient solutions on plant growth. Although we previously reported on the promotion of plant growth using a hydroponic culture system that continuously generated microbubbles, no data is available on the effects of intermittent microbubble generation on plant growth.

Although the microbubble generator used in this study was unable to generate as many microbubbles as the one used in our previous study (Park and Kurata 2009), it was nevertheless chosen because it is less expensive and can be easily installed into a hydroponic culture system. The objectives of this study were to investigate the effects of (1) different EC levels of microbubbles generated in nutrient solutions, and (2) periodic intermittent microbubble generation, on lettuce growth in a hydroponic culture system.

Materials and Methods

List of abbreviations

Dissolved oxygen (DO), Electrical conductivity (EC), Experiment (Exp.), Leaf dry weight (LDW), Leaf fresh weight (LFW), Leaf length (LL), Leaf number (LN), Leaf width (LW), Photosynthetic photon flux density (PPFD), Root dry weight (RDW)

Plant materials and culture conditions

Leaf lettuce seeds (*Lactuca sativa* L. 'Coslettuce') (Takii Seed Co., Tokyo, Japan) were sown in a vermiculite and peat mix (1:1) in a plant culture room (4 m × 5.7 m). Temperature was maintained at 21±1 °C (day) and 18 ±1 °C (night) using four air conditioners (SAP-V22B, SANYO Electric Co. Ltd., Tokyo, Japan). Fluorescent lamps (CREA1-FPL55EX-L, Iwasaki Electric Co. Ltd., Tokyo, Japan) provided a photosynthetic photon flux density (PPFD) of 180±25 μmol m^{-2} s^{-1} on the surface of the culture media. On day 14 after sowing, seedling roots were detached from the medium, washed, and wrapped in a urethane cube. One hundred seedlings were placed in a half-strength Yamazaki nutrient solution for Experiment (Exp.) I and II. After 1 week, 52 seedlings at four- or five-leaf stages were selected for three EC groups with microbubbles and one control with macrobubbles and transplanted 13 cm apart. Seedlings were cultured in four culture containers (46 cm long × 66 cm wide × 33 cm high) for 2 weeks at 21±1 °C (day) and 18±1 °C (night). Fluorescent lamps (CREA1-FPL55EX-L, Iwasaki Electric Co. Ltd., Tokyo, Japan) provided PPFDs of 163±21, 161±23, 163±21, or 163±22 μmol m^{-2} s^{-1} (mean±SD), averaged at 9 points at the canopy level for four conditions with a 12-h photoperiod. To maintain light uniformity, a shading net was placed between the light source and lettuce. To control the temperatures of the four nutrient solutions, tap water held in a container (33 cm long × 15 cm wide × 27 cm high) maintained at 10±1 °C with a cooling unit (Coolpipe 150LF, Taitec Co. Ltd., Saitama, Japan) was passed along spiral cooling pipes in the culture containers using underwater pumps (Rio90; Kamihata Fish Industry Co. Ltd., Hyogo, Japan). Temperatures of the nutrient solutions for all conditions were continuously maintained at 19±1 °C (day) and 16±1 °C (night). Microbubbles and macrobubbles were continuously generated at the bottom of the containers throughout the experiment.

EC and intermittent microbubble generation

Lettuce was cultured in containers filled with solution at 0.5, 1.0, or 2.0 dS m^{-1} for the microbubble conditions and 1.0 dS m^{-1} for the macrobubble condition (control) (Exp. I-A) using Yamazaki nutrient solution (YAMAZAKI 1978). To investigate the difference in plant growth between microbubble and macrobubble conditions under very low ion concentrations (Exp. I-B), lettuce was cultured in four containers filled with solutions at 0.5 or 1.0 dS m^{-1} for the microbubble and macrobubble conditions, respectively, under the same environmental conditions as described for Exp. I-A. The lettuce was grown in a DFT culture system with a periodic intermittent microbubble generation of 15, 30, or 60 min h^{-1} (control) for 2 weeks (Exp. II) and was cultured in containers filled with Otsuka nutrient solution (A prescription; Otsuka Chemical Co. Ltd., Tokyo) for leaf cultivation.

Microbubble and macrobubble generation

Microbubbles were generated using a microbubble generator (Besty; Febion Co. Ltd., Fukui, Japan) with a pump (PMD-1521B6E; Sanso Electric Co. Ltd., Hyogo, Japan). Fig. 1 shows a schematic diagram of the experimental apparatus used in this study. The microbubble generator consisted of a coaxial swirling flow-generating vane nozzle and vortex breakdown nozzle. Water entered the vane nozzle through a pump, which produced a swirling flow of liquid as a result of air being automatically passed through the center of the nozzle. The swirling flow was supplied to the vortex breakdown nozzle that constricts the swirling flow to generate microbubbles from a vortex breakdown section. Macrobubbles were generated through an aquarium aeration stone with an air pump (MV-70G; Enomoto Micro Pump Mfg. Co. Ltd., Tokyo, Japan) and had an average air flow rate of 1.0±0.1 L min^{-1}. Because the DO in the microbubble-generated nutrient solution without macrobubbles decreased gradually during the experiment, an average air flow rate of 0.2±0.1 L min^{-1} was supplied for the microbubble generation and 0.8±0.1 L min^{-1} was supplied by the aquarium aeration stones. The microbubble-generated nutrient solution had both microbubbles and macrobubbles. The distribution of bubbles sizes, as measured by a particle-counting spectrometer for liquids using a light-obscuration method, in the nutrient tank during the operation of the microbubble generator is shown in Fig. 2 (TAKAHASHI et al. 2007). Experimental conditions showed in Table 1.

Measurement and statistical analysis

DO, EC, and pH of the nutrient solutions were measured with a DO electrode (9520-10D with a D25 reading unit; Horiba Co. Ltd., Kyoto, Japan), an EC meter (B-173; Horiba Co. Ltd., Kyoto, Japan), and a pH electrode (6377-10D with a D21 reading unit; Horiba Co. Ltd., Kyoto, Japan), respectively, every 2 d throughout the experimental period. After the culture period, leaf fresh weight (LFW) and

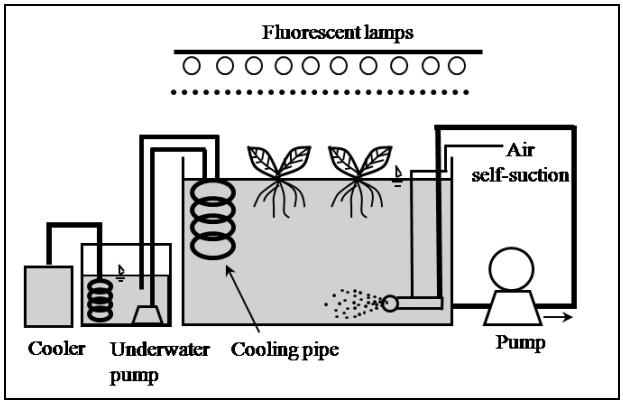


Fig. 1. Schematic diagram of the deep flow technique (DFT) hydroponic culture system with a microbubble generator and a cooling system.

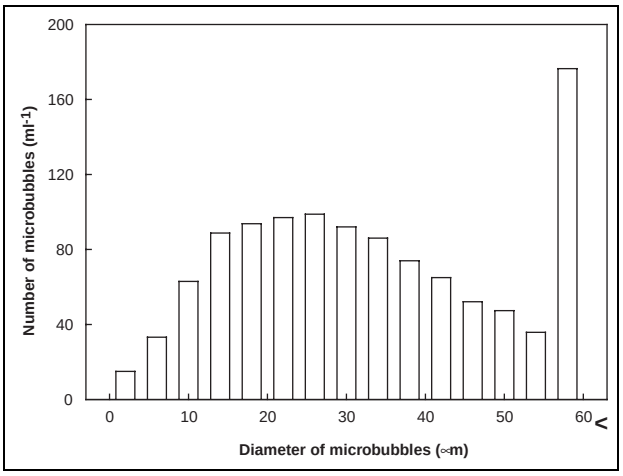


Fig. 2. Size distribution of microbubbles (MB) measured using a particle-counting spectrometer for liquids.

leaf number, length, and width were recorded. Leaves and roots were separated and placed in a dry oven (DX400; Yamato Scientific Co. Ltd., Tokyo, Japan) at 66 °C for 7 d. Leaf dry weight (LDW) and root dry weight (RDW) were also measured. Exp. I and II were repeated three times using a completely randomized design. Statistical analyses were performed using the JMP software (JMP; SAS Institute Inc., Cary, NC, USA), and involved analysis of variance (ANOVA) followed by Turkey's honestly significant difference (HSD) tests ($P<0.05$).

Results

Changes in pH, EC, and DO of the nutrient solution

Time courses of the changes in pH, EC, and DO in the nutrient solutions with microbubbles and macrobubbles were similar in Exp. I-A and I-B. The pH in all microbubbles and macrobubble conditions ranged similarly from 5.8 to 7.2 when Yamazaki nutrient solutions were applied in Exp. I (Fig. 3). In Exp. II the pH of the microbubble-generated Otsuka nutrient solutions decreased from 6.8 to 4.4 over 2 weeks, but the pH of the macrobubbles-generated solutions did not (Fig. 4). DO values in all conditions ranged from 8.2 to 8.7 mg L^{-1} for Exp. I (Fig. 3) and 9.1 to 9.6 mg L^{-1} for Exp. II (Fig. 4). EC values of the Yamazaki nutrient solutions and Otsuka nutrient solution on the last experimental day were not different from initial EC values (Fig. 3 and 4).

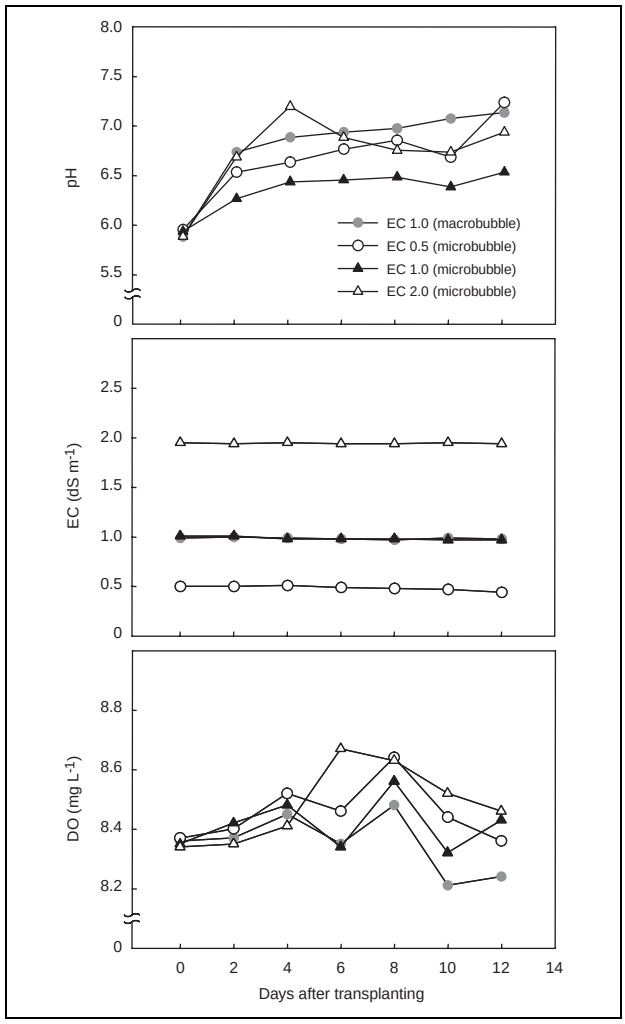


Fig. 3. Time course of the changes in pH, electrical conductivity (EC), and dissolved oxygen concentration (DO) in the Yamazaki nutrient solution (0.5, 1.0, and 2.0 dS m^{-1} EC) with microbubbles and in the nutrient solution (1.0 dS m^{-1} EC) with macrobubbles in a deep flow technique (DFT) hydroponic culture system for 2 weeks (Exp. I-A). Data shown are the mean values of three replications.

Table 1. Experimental conditions with 3 repetitions.

Experiment	Treatments		Plants per repetition
	EC (dS m^{-1})	Bubble generation intervals	
I-A	0.5 with macrobubbles 0.5, 1.0, and 2.0 with microbubbles		13
I-B	0.5 and 1.0 with macrobubbles 0.5 and 1.0 with microbubbles		13
II		60 min h^{-1} with macrobubbles 15, 30, and 60 min h^{-1} with microbubbles	13

Plant growth

The LFWs of lettuce treated with 0.5 and 1.0 dS m^{-1} EC microbubbles were 1.54 and 1.43 times greater than those treated with macrobubbles (Table 2). However, the LFW was not significantly different between the 2.0 dS m^{-1} EC microbubble condition and the macrobub-

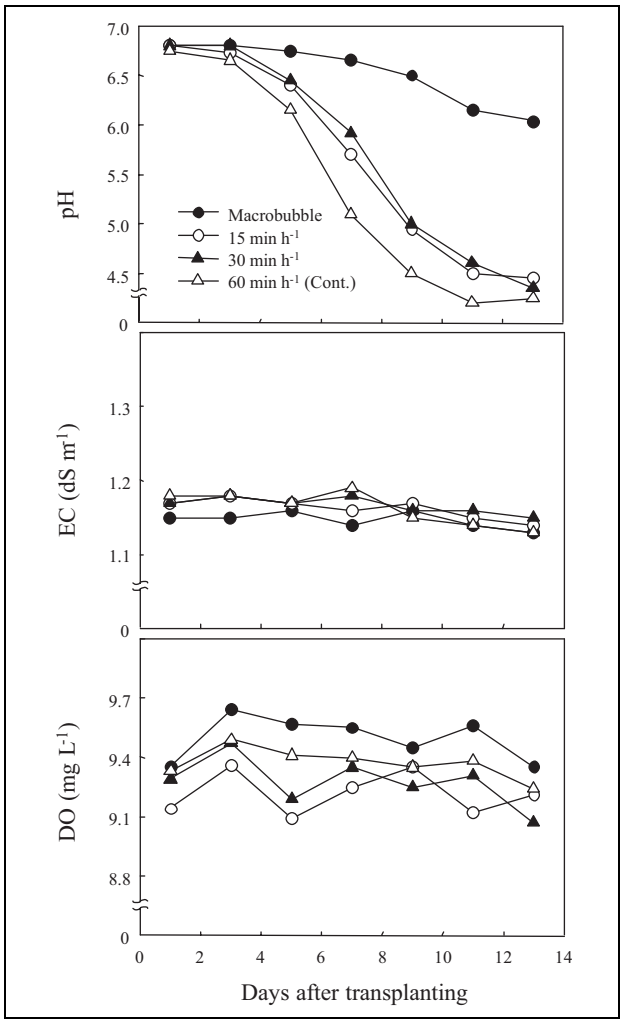


Fig. 4. Time course of the changes in pH, electrical conductivity (EC), and dissolved oxygen concentration (DO) in the Otsuka nutrient solution with continuous macrobubbles generation or periodic intermittent microbubbles generation [15, 30, and 60 min h^{-1} (Cont.)] in a deep flow technique (DFT) hydroponic culture system for 2 weeks (Exp. II). Data shown are mean values of three replications.

ble condition (Table 2). Although the reduction in the LFW was significantly greater in the 2.0 dS m^{-1} EC microbubble condition, there was no difference in LDW among the three microbubble conditions (Table 2). Leaf color of lettuce grown in the 2.0 dS m^{-1} EC microbubble condition was dark green and leaf was thicker than those treated with 0.5and 1.0 dS m^{-1} EC microbubble. In our study, there was no significant difference in lettuce leaf number and leaf length among all conditions, while leaf width was significantly greater under the 0.5 and 1.0 dS m^{-1} EC microbubble conditions than those grown under the 2.0 dS m^{-1} EC microbubble and the 1.0 dS m^{-1} EC macrobubble conditions (Table 2).

The LFWs of lettuce treated with microbubbles for 15 and 30 min h^{-1} were 13 and 4 % significantly greater, respectively, than those treated for 60 min h^{-1} (Fig. 5), showing no significant difference. Although the LDWs of lettuce treated with microbubbles were greater than those treated with macrobubbles, there was no significant difference in the LDWs of lettuce treated with microbubbles for 15, 30, and 60 min h^{-1} (Fig. 5). Leaf number of lettuce treated with microbubbles for 15 min h^{-1} was significantly greater than that treated with macrobubbles. The RDWs did not differ significantly among any of the conditions.

Discussion

Changes in pH, EC, and DO of the nutrient solution

Similar trends in pH, EC, and DO between macrobubble and microbubble-generated hydroponic culture systems with the nutrient solutions used for lettuce cultivation were reported by Park and Kurata (2009). pH in all the treatment for Exp. I ranged from 5.8 to 7.0. Radical decrease in pH of the microbubble treatments for Exp. II was shown, whereas plant growth was greater than that of the macrobubble treatment which showed stable change in pH from 6.8 to 6.3. A decrease in pH in nutrient solutions indicates a higher uptake of positively charged ions than negatively charged ions by roots. More absorption of positive ions by the plant (such as potassium, calcium, magnesium, and ammonium) caused roots to discharge hydrogen ions to balance the rate of cations and anions in plant body. The content of each ion in the Otsuka nutrient solution is greater than that in the Yamazaki nutrient solution: N-18.6, P-5.1, K-8.6, Ca-8.2, and Mg-3.0 me L^{-1} and N-6.5, P-1.5, K-4, Ca-2, and Mg-1 me L^{-1}, respectively. Otsuka nutrient solution with 1.2 dS m^{-1} EC has much more positive ions than Yamazaki (around 3 times stronger). As electrically negative charges (ζ potential) develop on the surface of microbubbles under a wide range of pH conditions, positively charged ions accumulate around the microbubbles to create an electrical double layer (Takahashi 2005). Microbubbles gather easily positive ions around their surface under the higher concentration condition. Then, absorption rate of positive ions by roots increases, compared to low positive ions concentration condition. Therefore more absorption of positive ions by roots may discharge more hydrogen ions to balance ionic state in plant body.

Takahashi et al. (2003) reported increasing DO concentrations in microbubble-generated water solutions because of the excellent gas dissolution ability of microbubbles. However, DO concentrations in our nutrient solutions did not increase because the air supplied for microbubble generation was only 20 % of that used for macrobubble generation. As the temperature of the nutrient solution increases, the DO concentration decreases. The difference in DO concentrations on the same day contributed to the <1 °C difference in temperature.

Plant growth

We predicted that the growth of lettuce in the 0.5 dS m^{-1} EC macrobubble condition will be less than that of lettuce grown in the 1.0 dS m^{-1} EC macrobubble condition, but there was no statistical difference in any of the growth parameters between the 0.5 and 1.0 dS m^{-1} EC conditions in the microbubble- and macrobubble-generated conditions, respectively. The LFW of lettuce grown under the

Table 2. Leaf fresh weight (LFW), leaf number (LN), leaf length (LL), leaf width (LW), leaf dry weight (LDW), and root dry weight (RDW) of lettuce plants grown for 2 weeks in deep flow technique (DFT) hydroponic culture system with microbubbles generated at electrical conductivities (ECs) of 0.5, 1.0, and 2.0 dS m^{-1} or macrobubbles generated at an EC of 1.0 dS m^{-1} in the nutrient solution.

Experiment		EC (dS m^{-1})	LFW (g)	LN	LL (cm)	LW (cm)	LDW (g)	RDW (g)
I-A	Macrobubbles	1.0	12.8 ± 0.57 b	17.9 ± 0.45	13.8 ± 0.48	7.7 ± 0.10 b	0.66 ± 0.035 b	0.10 ± 0.004 b
	Microbubbles	0.5	19.8 ± 0.77 a	19.1 ± 0.35	14.1 ± 0.22	9.3 ± 0.15 a	0.90 ± 0.047 a	0.12 ± 0.003 a
		1.0	18.3 ± 0.78 a	19.0 ± 0.32	14.6 ± 0.24	8.7 ± 0.11 a	0.91 ± 0.035 a	0.12 ± 0.004 a
		2.0	14.8 ± 0.49 b	18.6 ± 0.33	14.2 ± 0.18	8.2 ± 0.18 b	0.87 ± 0.029 a	0.12 ± 0.005 a
I-B	Macrobubbles	0.5	11.7 ± 0.62 b	17.2 ± 0.18 c	12.7 ± 0.19 b	8.2 ± 0.14 b	0.62 ± 0.025 b	0.11 ± 0.004 ab
		1.0	12.1 ± 0.73 b	17.7 ± 0.25 bc	13.6 ± 0.24 ab	8.3 ± 0.20 b	0.63 ± 0.045 b	0.10 ± 0.006 b
	Microbubbles	0.5	18.1 ± 0.63 a	18.9 ± 0.13 ab	13.9 ± 0.18 a	9.3 ± 0.14 a	0.87 ± 0.043 a	0.13 ± 0.005 a
		1.0	18.9 ± 1.44 a	19.0 ± 0.35 a	14.2 ± 0.33 a	9.3 ± 0.24 a	0.88 ± 0.063 a	0.13 ± 0.006 a

Data represent the means (± se).
Means followed by the same letter within a column are not significant different by Tukey's hsd test ($P < 0.05$).

2.0 dS m^{-1} EC microbubble condition did not increase compared to other microbubble conditions. While some studies have reported reduced tomato fruit weights under high EC (Cuartero and Fernández-Munoz 1999; Fanasca et al. 2007), Wu and Kubota (2008) found that high EC treatment (4.5 dS m^{-1}) did not significantly affect the fresh weight of tomatoes compared to normal EC treatment (2.3 dS m^{-1}). Microbubbles generated in nutrient solution promote plant growth because their larger specific surface area (compared to macrobubbles) has greater negative electronic charges, which may help the roots to absorb positive ions (Park and Kurata 2009). Although the reasons for growth decrease of lettuce under 2.0 dS m^{-1} with microbubbles are still under investiga-

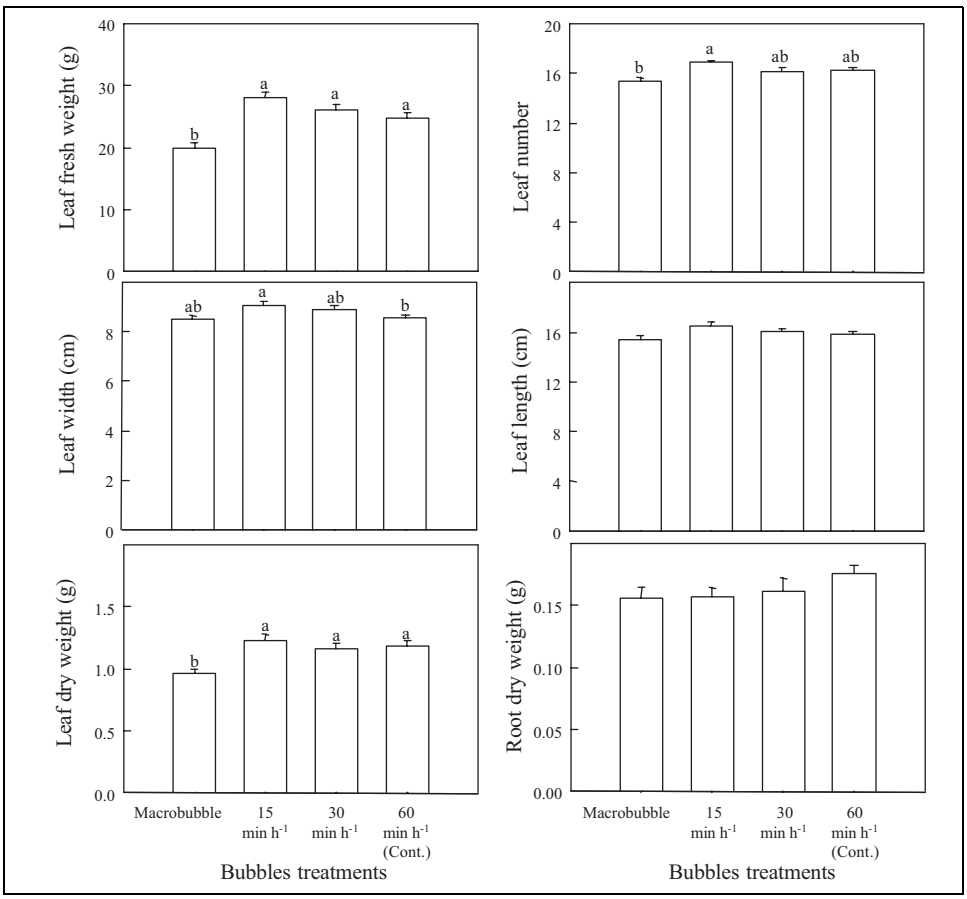


Fig. 5. Leaf fresh weight, leaf number, leaf length, leaf width, leaf dry weight, and root dry weight of leaf lettuce grown under continuous macrobubbles generation or periodic intermittent microbubble generations [15, 30, and 60 min h^{-1} (Cont.)] in a deep flow technique (DFT) hydroponic culture system for 2 weeks. Data represent the means (±SE). Means with different letters are significantly different by Turkey's HSD test ($P < 0.05$).

tion, higher ion concentration with microbubbles in nutrient solution may cause irregular ion absorption by roots and this may work as a stress to root system.

Lettuce growth treated with periodic generation of microbubbles for 15, 30, and 60 min h^{-1} was not significantly different regardless of significant difference between microbubble and macrobubble treatments. No difference in growth of lettuce between 15 and 60 min h^{-1} treatments means that the efficiency of periodic generation of microbubbles on roots development was not different. Retention time of microbubbles will quite be longer than that of macrobubbles. The retention time of microbubbles in some solution greatly increase compared to macrobubbles due to their low floating velocity and ionic double layer around their surface (TAKAHASHI 2005). Therefore, large numbers of generated microbubbles were not easily disappeared in nutrient solution with no break, or have some stable status in some special solution conditions. Although studying retention time of microbubbles in nutrient solution is needed, based on these results, microbubbles generated periodically in a microbubble generation system can be supplied to many culture beds. It is likely that these characteristics of microbubbles, large number of microbubbles, stable status with double layer, and having more retention time, can contribute plant growth increase in lettuce hydroponics culture system. These results indicate that the microbubble generation in nutrient solutions can promote lettuce growth and intermittent microbubble generation for 30 min h^{-1} or less can be adapted effectively in hydroponic culture systems.

Implementation

To apply this microbubbles technology to hydroponics culture system, 294 KPa (3 kg cm^{-2}) or over tap-water pressure is needed by a pump or gravity. Microbubbles can be generated in a nutrient tank with short irrigation piping systems, otherwise microbubble generator can be installed at the end of irrigation piping system, right before water supply to culture bed. Retention time of microbubble without shrinking and disappearing would be very important factor to promote plant growth.

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Received January 10, 2010 / Accepted August 02, 2010

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