Application of a Growth Model to Validate the Effects of an Ultrafine-bubble Nutrient Solution on Dry Matter Production and Elongation of Tomato Seedlings

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To clarify the effect of ultrafine bubbles (UFBs) on the growth of tomato seedlings, we investigated elongation growth and dry matter production by analysing growth under different assimilation conditions and by modelling. The leaf area enlargement rate of plants grown with UFB nutrient solution increased and the specific leaf area (SLA) decreased at 18 days after sowing (DAS) relative to those grown without UFB solution. Thus, UFBs increased both leaf area and leaf thickness. UFB significantly increased the relative growth rate (RGR) and net assimilation rate (NAR) at 18 DAS, but there was no significant difference in SLA, RGR, and NAR between treatments at 25 DAS. These results were used to model plant growth with and without UFB treatment. In a second experiment, UFB treatment increased aboveground dry weight under a low-assimilation condition, but had no significant effect under a high-assimilation condition. Our model supported these results. It was also implied that UFB treatment affected leaf area expansion, but not dry matter production. Although the values predicted by the model were slightly lower than observed, it was possible to predict the effect of UFB treatment on plant growth with high accuracy.

Key Words: aboveground dry weight, leaf area index, leaf expansion, modelling, specific leaf area.

Introduction

Fine bubbles (FBs) can act as surfactants, apply impact pressure, maintain antioxidant power, and have physiological activity (Kido, 2016; Nakano, 2016). Thus, FBs are an innovative technology that could be useful in a wide variety of industries in Japan. FBs include microbubbles (MBs), with a diameter of 1 to 100 μm, and ultrafine bubbles (UFBs), with a diameter of ≤ 1 μm (Kido, 2016). Although MBs in water disappear after a few minutes, UFBs can persist for several months (Nishihara and Maeda, 2014). Because gas can be confined in water by the UFB technique, many applications of UFBs in industry are anticipated (Nishihara and Maeda, 2014), and the use of FBs is now being investigated in agricultural production. Himuro (2016) reported that FBs increased yield in strawberry (11%), snap pea (14%), eggplant (22%), tomato (24%), and Japanese pear (25%). Minagawa et al. (2016) reported that the yield of spinach grown in a 1/10 nutrient solution with MBs was comparable to that grown in a full nutrient solution under a deep-flow technique, indicating that MB application could reduce fertilizer use by 90%. Yamamoto et al. (2016, 2017) reported that two-week-old tomato plants fertigated with UFB nutrient solution had larger stem diameters, longer roots, and more cytokinin than those fertigated without a UFB solution. By contrast, Nakano et al. (2010) reported that MB nutrient solution did not increase the growth of drip-irrigated roses. Although the effects of MBs and other FBs may differ depending on the species and irrigation method, there are few reports on their mechanism of action or regarding the conditions under which they are most effective. In addition, there have been no reported physiological or ecological
studies on how FB treatment increases yields. Although UFB treatment improved the growth and yield of several crops, as described above, it is not clear whether UFBs affect elongation growth, photosynthesis, or both.

Growth analysis and modelling are useful tools for investigating plant growth under different conditions. We showed that when l-α-aminoxy-β-phenyl propionic acid, an auxin biosynthesis inhibitor, was supplied to tomato seedlings, elongation growth was suppressed, but the net assimilation rate was not affected (Higashide et al., 2014). Both observation and growth modelling showed that the effect of the inhibitor depended on the environmental conditions, even if there was no difference in photosynthetic ability per unit leaf area. In this study, we therefore sought to clarify the effects of UFB treatment on growth and dry matter (DM) production of tomato seedlings using growth analysis and modelling as described by Higashide et al. (2014) Using this model, we tried to predict the growth of seedlings grown with or without UFB treatment under different day length conditions. We then validated the model by comparing its predictions with data from a second, independent experiment.

**Materials and Methods**

1. **Effect of UFB nutrient solution on growth of tomato seedlings**

   The Japanese tomato cultivar ‘Momotaro York’ (Takii Seed, Kyoto, Japan) was grown in these experiments. For Experiment 1, seeds were sown on 13 June 2016 in 72-well seed trays (30 cm × 60 cm) filled with nursery soil and germinated in a germination-forcer (WR-60CHL-S; Keibun, Akitakada, Japan). After three days, the trays were placed in a seedling growth chamber (Seedling Terrace; MKV Dream, Tokyo, Japan). The seedlings were illuminated with fluorescent lamps using a 16-h day length and a photosynthetic photon flux density (PPFD) of 337–351 μmol·m⁻²·s⁻¹ (P = 0.36), and were grown in 1000 μmol·mol⁻¹ CO₂ at air temperatures of 25°C (day) and 15°C (night).

   Every day, the seedlings were fertilized with 500 mL/tray of nutrient solution (OAT A treatment; OAT Agrio, Tokyo, Japan) with UFBs (UFB+) or without UFBs (UFB−). The solution consisted of 7.2 mM NO₃⁻, 3.3 mM K⁺, 3.2 mM Ca²⁺, 1.2 mM Mg²⁺, 2.0 mM H₂PO₄⁻, 1.0 mg·L⁻¹ Fe, 0.58 mg·L⁻¹ Mn, 0.58 mg·L⁻¹ B, 0.03 mg·L⁻¹ Zn, 0.01 mg·L⁻¹ Cu, and 0.01 mg·L⁻¹ Mo, and was adjusted to an electrical conductivity of 1.0 dS·m⁻¹. Each day, about 180 L of UFB nutrient solution for the following day was prepared in a UFB generator (Ultrafine GaIF, FZ1N-10; IDEC Corporation, Osaka, Japan) with an eight-hour operation time. The UFB concentrations during this experiment were measured with a NanoSight NS500 instrument (Malvern Instruments Ltd, Worcestershire, UK) using a 50 mW 635 nm laser and a scientific complementary metal-oxide-semiconductor (sCMOS) camera. Video files were acquired and analysed using the instrument software version NTA 3.2. As expected, the UFB concentration in the UFB+ treatment (2.87 × 10⁸ particles·mL⁻¹) was significantly higher than that in the UFB− treatment (1.78 × 10⁸ particles·mL⁻¹; Fig. 1A). We inferred that the background counts in the latter case were attributable to impure small particles in raw water, rather than UFBs from a comparison of the number density profiles. The dissolved oxygen concentration and solution temperature did not differ significantly between treatments: 7.87 mg·L⁻¹ and 23.2°C in the UFB+ solution and 7.79 mg·L⁻¹ and 22.9°C in the UFB− solution (Fig. 1B, C).

   The experiment used a randomized complete block design with three blocks per treatment. Each block contained 15 plants with nine surrounding plants. Seedlings were sampled at 11, 18, and 25 days after sowing (DAS). At each time point, the leaf area and the fresh and dry weight of leaves and stems of 10 randomly collected plants per block per treatment were measured. The leaf areas were measured with image analysis software (Lia 32) using digital photographs (EOS Kiss X 3; Canon, Tokyo, Japan) at 11 DAS, and with an area meter (LI-3100; LI-COR, Lincoln, NE, USA) at 18 and 25 DAS. The aboveground dry weight was measured.
after drying at 105°C for 72 h. The relative growth rate (RGR) and net assimilation rate (NAR) were calculated as:

\[
RGR = \frac{\ln(W_f) - \ln(W_i)}{(t_2 - t_1)} \quad \text{Eqn. 1}
\]
\[
NAR = \frac{(W_f - W_i)}{(A_f - A_i) \cdot \ln(A_f) / (t_2 - t_1)} \quad \text{Eqn. 2}
\]

where \(W_i\) and \(W_f\) are the aboveground dry weight (g) at \(t_1\) and \(t_2\), respectively, and \(A_i\) and \(A_f\) are the leaf area (m²) at \(t_1\) and \(t_2\), respectively.

The rate of leaf area enlargement was calculated as:

\[
\text{Leaf area enlargement} = \frac{\ln(A_f) - \ln(A_i)}{\ln(A_f)} \quad \text{Eqn. 3}
\]

2. Modelling of DM production and elongation of tomato seedlings grown in UFB+ or UFB−

We modelled DM production and elongation of seedlings according to Higashide et al. (2014). The increase in leaf area index (LAI, m²·m⁻²) can be described as:

\[
dA/dt = v_1 \cdot dM/dt \quad \text{Eqn. 4}
\]

where \(A\) is LAI (m²·m⁻²), \(M\) is DM weight per unit area (g·m⁻²), and \(v_1\) is the rate of increase in LAI per unit DM (m²·g⁻¹). We described the influence of UFB treatment on the leaf area expansion as elongation growth by the following equation:

\[
dA/dt = u \cdot v_1 \cdot dM/dt \quad \text{Eqn. 5}
\]

where \(u\) represents the elongation coefficient of leaf area on UFB. Since light interception by plants is determined by LAI and the light extinction coefficient within the canopy, DM production by plants can be described as:

\[
dM_p/dt = LUE \cdot (1 - e^{-k \cdot LAI}) \cdot S_i \quad \text{Eqn. 6}
\]

where \(M_p\) is potential DM weight (i.e., with no down-regulation of photosynthesis), LUE is the light-use efficiency (g·mol⁻¹·PPFD), \(k\) is the light-extinction coefficient, and \(S_i\) is PPFD (mmol·m⁻²·s⁻¹). Plants grown under elevated CO₂ or high light levels show a down-regulation of photosynthesis owing to an excessive accumulation of photo assimilate in the leaves, which reduces DM production. The potential DM production represents production under the assumption of no restriction by down-regulation. We assumed in our modelling that DM production would be reduced by photosynthetic assimilate accumulation, and that the assimilate reservoir and its rate of use were determined by plant size. Accordingly, the upper limit of the growth rate in our model may increase with increasing plant weight. The upper limit of DM production (\(l\), g·m⁻²·d⁻¹) can be described as:

\[
l = m \cdot M \quad \text{Eqn. 7}
\]

where \(m\) is the coefficient for the upper limit that is related to the reservoir size and to the rate of assimilate use (g·g⁻¹·d⁻¹), and \(M\) is the dry weight (g·m⁻²). We assumed that actual DM production (\(M\); g·m⁻²) could be described as follows:

\[
\text{If } l \cdot \left(1 - e^{-dM_p/dt}\right) \geq dM_p/dt, \quad dM_p/dt = dM_p/dt \quad \text{Eqn. 8}
\]
\[
\text{If } l \cdot \left(1 - e^{-dM_p/dt}\right) < dM_p/dt, \quad dM_p/dt = l \cdot \left(1 \quad \text{Eqn. 9}
\]

Based on Higashide and Heuvelink (2009) and Higashide et al. (2014), we set \(k = 0.8\) and \(m = 0.260\) (g·g⁻¹·d⁻¹) in the above equations. According to the results of Experiment 1, we set \(u = 1.02\), LUE = 1.063 (g·mol⁻¹), and \(v_1 = 0.026\) (m²·g⁻¹).

3. Effect of UFB nutrient solution on growth of tomato seedlings at different day lengths and validation of the model

To validate the model (Eqns. 3–9) with independent data, we conducted Experiment 2, which compared the growth of tomato seedlings with/without UFB treatment under different daily amounts of light interception (i.e., different assimilation conditions). Since tomato seedlings grown under artificial light change morphologically if the light intensity and wavelength are varied (Nanya et al., 2015), we changed the day length to alter the cumulative daily light interception, thus avoiding the development of spindly seedlings. Except for day length, the conditions and design were similar to those used in Experiment 1. Seeds of ‘Momotaro York’ were sown in trays on 12 September and 11 October 2016. At 3 DAS, the seedlings were placed in a growth chamber under respective day lengths of 16 and 18 h to achieve a respective daily cumulative light interception of 16.0 and 28.17 mol·m⁻²·d⁻¹. At 21 DAS, the seedlings were sampled and measured as in Experiment 1.

We used Eqns. 3–9 to predict the aboveground dry weight and LAI of tomato seedlings with or without UFB treatment at 21 DAS. The coefficients used for Eqns. 3–9 were the same as those used in the modelling, and the light conditions were the same as those used in Experiment 2. We calculated Pearson’s coefficient of correlations between the observed and predicted values of aboveground dry weight and LAI at 21 DAS and tested the correlation.

Results

1. Effect of UFB nutrient solution on growth of tomato seedlings

At 18 DAS, there was no significant difference in leaf number, stem length, or leaf area between UFB+ and UFB− treatments (Table 1). Aboveground dry weight and DM content were significantly higher in UFB+ than in UFB−. At 25 DAS, there was no significant difference in the number of leaves between treatments; however, stem length, leaf area, aboveground dry weight, and DM content were significantly higher in UFB+ than in UFB−.
At 18 DAS, RGR, NAR, and leaf mass per area (LMA) were significantly higher in UFB+ than in UFB− (Table 2). Leaf area ratio (LAR) and SLA were significantly smaller in UFB+ than in UFB−. There was no significant difference in the leaf mass ratio (LMR) between UFB+ and UFB−. However, at 25DAS, there was no significant difference in RGR, NAR, LAR, LMR, SLA, and LMA between treatments. Only leaf enlargement was significantly higher in UFB+ than in UFB−.

### Table 1. Effect of UFB treatment on the number of leaves, stem length, leaf area, and aboveground dry weight of young tomato plants at 18 and 25 days after sowing.

| Days after sowing | Treatment  | Leaf number | Stem length | Leaf area | Aboveground dry weight | Dry matter content |
|-------------------|------------|-------------|-------------|-----------|------------------------|--------------------|
|                   |            | leave/plant | cm/plant    | cm²/plant | g/plant                | g·g⁻¹               |
| 18                | UFB−       | 4.6         | 5.53        | 33.8      | 0.161                  | 0.098              |
|                   | UFB+       | 4.8         | 5.55        | 36.4      | 0.201                  | 0.101              |
|                   | t-test     |             |             |           | **                     |                    |
| 25                | UFB−       | 6.5         | 10.1        | 108.3     | 0.497                  | 0.091              |
|                   | UFB+       | 6.6         | 10.9        | 116.0     | 0.541                  | 0.114              |
|                   | t-test     |             |             |           | NS                    |                    |

z: UFB−, nutrient solution without UFBs; UFB+, nutrient solution with UFB.
y: NS, * and ** indicate non-significance or significance at the 0.05 and 0.01 levels by t-test, respectively (n=30).

### Table 2. Effect of UFB treatment on the relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), leaf mass ratio (LMR), specific leaf area (SLA), leaf mass per area (LMA), and leaf area enlargement of young tomato plants at 18 and 25 days after sowing.

| Days after sowing | Treatment  | RGR  | NAR  | LAR  | LMR  | SLA  | LMA  | Leaf area enlargement |
|-------------------|------------|------|------|------|------|------|------|-----------------------|
|                   |            | g·g⁻¹·d⁻¹ | g·m⁻²·d⁻¹ | m²·g⁻¹ | g·g⁻¹ | m²·g⁻¹ | g·m⁻² | %                     |
| 18                | UFB−       | 0.201 | 9.61 | 0.022 | 0.835 | 0.026 | 39.2 | 100                   |
|                   | UFB+       | 0.236 | 13.11 | 0.018 | 0.836 | 0.022 | 46.2 | 102                   |
|                   | t-test     |      |     |     |     |      |      |                       |
| 25                | UFB−       | 0.213 | 9.79 | 0.022 | 0.816 | 0.027 | 37.3 | 100                   |
|                   | UFB+       | 0.220 | 10.30 | 0.022 | 0.811 | 0.027 | 37.7 | 102                   |
|                   | t-test     |      |     |     |     |      |      |                       |

z: UFB−, nutrient solution without UFBs; UFB+, nutrient solution with UFB.
y: NS, * and ** indicate non-significance or significance at the 0.01 levels by t-test, respectively (n=30).

### Table 3. Effect of UFB treatment on the number of leaves, stem length, leaf area, and aboveground dry weight of young tomato plants under different day lengths at 21 days after sowing.

| Day length | Treatment  | Leaf number | Stem length | Leaf area | Aboveground dry weight | Dry matter content |
|------------|------------|-------------|-------------|-----------|------------------------|--------------------|
|            |            | leave/plant | cm/plant    | cm²/plant | g/plant                | g·g⁻¹               |
| 16-h       | UFB−       | 4.0         | 6.36        | 33.7      | 0.165                  | 0.092              |
|            | UFB+       | 4.1         | 6.69        | 42.8      | 0.187                  | 0.089              |
|            | t-test     |             |             |           | NS                    |                    |
| 18-h       | UFB−       | 5.2         | 6.2         | 61.6      | 0.224                  | 0.079              |
|            | UFB+       | 5.2         | 6.7         | 62.6      | 0.226                  | 0.079              |
|            | t-test     |             |             |           | NS                    |                    |

z: UFB−, nutrient solution without UFBs; UFB+, nutrient solution with UFB.
y: NS, * and ** indicate non-significance or significance at the 0.05 and 0.01 levels by t-test, respectively (n=30).
enlargement of UFB+ were higher than those of UFB−; the LMA of UFB+ was lower than that of UFB− (Table 4). There was no significant difference in NAR and LMR between UFB+ and UFB−. With an 18-h day length, there was no significant difference in the growth analysis parameters between UFB+ and UFB−.

3. Validation of the model and prediction of tomato seedling growth with/without UFB treatment under different light conditions.

Although predicted DM production was higher in UFB+ with a 16-h day length than in UFB− at both 18 and 21 DAS, there was no predicted difference with an 18-h day length (Table 5). As predicted by the model, the observed aboveground dry weight and LAI were significantly higher with an 18-h day length than with a 16-h day length, regardless of UFB treatment (Fig. 2). Both parameters were significantly higher in UFB+ with a 16-h day length, but there was no effect of UFB treatment with an 18-h day length. Pearson’s correlations between the observed and predicted dry weights ($R^2 = 0.85; P < 0.05$) and LAIs ($R^2 = 0.95; P < 0.01$) were significant and positive. However, all of the predicted values were lower than the observed values, except for LAI in UFB− with a 16-h day length, which had almost equal observed and predicted values. The regression lines of the predicted against observed values were $y = 0.94x$ for aboveground dry weight and $y = 0.86x$ for LAI.

**Discussion**

In the present study, UFB promoted DM production, i.e. above ground dry weight, at 18 DAS in Experiment 1 (Table 1), and with a 16-h day length in Experiment 2 (Table 3). However, at 25 DAS in Experiment 1, and with an 18-h day length in Experiment 2, DM production was not affected by UFB (Tables 1 and 3). Based on the growth analysis, at 18 DAS in Experiment 1, and with a 16-h day length in Experiment 2, RGR was increased by UFB. Since the NAR multiple LAR i.e. LMR-SLA, equals RGR, the high RGR due to UFB was mainly caused by the high NAR at 18 DAS in Experiment 1 (Table 2), but by high SLA with a 16-h day length in Experiment 2 (Table 3). It was previously reported that an increase in leaf thickness improves the photosynthetic rate per unit leaf area (Fan et al., 2013).

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### Table 4. Effects of UFB treatment on the relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), leaf mass ratio (LMR), specific leaf area (SLA), leaf mass per area (LMA), and leaf area enlargement of young tomato plants under different day lengths at 21 days after sowing.

| Day length | Treatment $^z$ | RGR $^{g \cdot g^{-1} \cdot d^{-1}}$ | NAR $^{g \cdot m^{-2} \cdot d^{-1}}$ | LAR $^{m^{-2} \cdot g^{-1}}$ | LMR $^{g^{-1}}$ | SLA $^{g \cdot m^{-2}}$ | LMA $^{g \cdot m^{-2}}$ | Leaf area enlargement $^{\%}$ |
|------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|
| 16-h       | UFB−           | 0.188           | 9.24            | 0.020           | 0.776           | 0.026           | 38.2            | 100             |
|            | UFB+           | 0.207           | 9.20            | 0.023           | 0.765           | 0.030           | 33.9            | 115             |
|            | t-test         | **              | NS              | **              | NS              | **              | **              | NS              |
| 18-h       | UFB−           | 0.240           | 8.75            | 0.028           | 0.767           | 0.036           | 27.9            | 100             |
|            | UFB+           | 0.241           | 8.72            | 0.028           | 0.764           | 0.036           | 27.6            | 101             |
|            | t-test         | NS              | NS              | NS              | NS              | NS              | NS              | NS              |

*z: UFB−, nutrient solution without UFBs; UFB+, nutrient solution with UFB. 
y: NS and ** indicate non-significance or significance at the 0.01 levels by $t$-test, respectively (n=30).

### Table 5. Prediction of dry matter production and crop growth rate (CGR) of tomato plants grown with 16 and 18 h day lengths with/without UFB at 18 and 21 days after sowing.

| Days after sowing | Day length | Treatment $^z$ | $dM/dt$ $^{g \cdot m^{-2}}$ | $1/(1-e^{-dM/dt})$ $^{g \cdot m^{-2}}$ | $dM/dt'$ $^{g \cdot m^{-2}}$ | CGR $^{g \cdot m^{-2} \cdot d^{-1}}$ |
|------------------|------------|----------------|-----------------|-----------------|-----------------|-----------------|
| 18               | 16-h       | UFB−           | 5.88            | 7.50            | 5.88            | 4.35            |
|                  |            | UFB+           | 5.94            | 7.51            | 5.94            | 4.38            |
|                  | 18-h       | UFB−           | 12.82           | 9.03            | 9.03            | 6.60            |
|                  |            | UFB+           | 12.95           | 9.03            | 9.03            | 6.60            |
| 21               | 16-h       | UFB−           | 9.72            | 13.08           | 9.72            | 7.78            |
|                  |            | UFB+           | 9.86            | 13.17           | 9.86            | 7.89            |
|                  | 18-h       | UFB−           | 21.64           | 18.07           | 18.07           | 13.20           |
|                  |            | UFB+           | 21.81           | 18.07           | 18.07           | 13.20           |

*z: UFB−, nutrient solution without UFBs; UFB+, nutrient solution with UFB. 
y: If $1/(1-e^{-dM/dt}) \geq dM/dt$, then $dM/dt = dM/dt$. If $1/(1-e^{-dM/dt}) < dM/dt$, then $dM/dt = 1/(1-e^{-dM/dt})$. (Eqn. 8 and 9).
so it could be inferred that the high NAR at 18 DAS was caused by the increase in leaf thickness. Although the reason for the high RGR remains unclear, NAR, and SLA were no different with UFB at 25 DAS in Experiment 1 (Table 2), and with an 18-h day length in Experiment 2 (Table 4). These results showed that UFB promoted growth of tomato seedlings only at the beginning stage, but not at the later stage.

Stem length and leaf area were increased significantly by UFB at 25 DAS in Experiment 1 (Table 1), and with a 16-h day length in Experiment 2 (Table 3). Leaf area enlargement was significantly increased by UFB treatment at both 18 and 25 DAS, and with a 16-h day length in Experiment 2. These results show that UFB treatment promoted elongation growth of the seedlings. Yamamoto et al. (2016, 2017) reported that two-week-old tomato plants fertigated with UFB nutrient solution had larger stem diameters, longer roots, and more cytokinin than those fertigated without UFB solution. These reports are consistent with our findings that UFBs promote elongation growth.

There was no difference in leaf area at 18 DAS, LAR and SLA were reduced, and LMA was increased, by UFB+ (Table 2). These results indicate that UFB treatment increased leaf thickness and leaf volume. Thus, UFB treatment may promote not only expansion of leaf area, but also increase leaf thickness. At 25 DAS in Experiment 1 and with an 18-h day length in Experiment 2, there was no significant difference in either SLA or NAR between UFB+ and UFB−. Thus, there was no difference in NAR because there was no difference in leaf thickness (SLA), and it could also be inferred that there was no UFB treatment influence on the assimilation rate at this time point. Taking the above observations together, UFB treatment promoted elongation growth, leaf thickness, leaf area, and stem elongation of tomato seedlings, although it was unclear whether or not NAR was directly improved by UFB.

Next, we verified the model assuming only the effect on elongation growth. With a 16-h day length (low-assimilation condition), both DM production and LAI were higher in UFB+ than in UFB− (Fig. 2). However, with an 18-h day length (high-assimilation condition), neither DM production nor LAI differed significantly between UFB+ and UFB−. These results were also supported by modelling (Table 5). Plants grown under high light levels show a down-regulation of photosynthesis (Faria et al., 1996). In this phenomenon, the photosynthetic rate may decrease due to an excessive accumulation of photo assimilate in the leaves (Paul and Pellny, 2003), leading to decreased dry matter production. We assumed the limitation of actual DM production in Eqn. 8 and 9. Although the potential DM production with an 18-h day length was higher than that with a 16-h day length, actual DM production may be maximal with an 18-h day length. These results imply that UFB treatment promoted leaf area expansion and in turn DM production under the low-assimilation condition. However, the mechanisms leading to leaf area expansion and DM production may have been maximal under the high-assimilation condition. These results also imply that UFB treatment did not affect DM production, even when it promoted leaf area expansion.

Minagawa et al. (2016) reported a 90% saving in fertilizer when MB nutrient solution was used in spinach production. Since leaf vegetables such as spinach have a short life from sowing to harvesting, FB treatment could contribute to an expansion of leaf area and thus an increase in yield. Although increases in yield of FB-treated tomato, eggplant, and strawberry have been reported (Himuro, 2016), our results did not demonstrate consistent enhancement of DM production in UFB-treated tomato plants. Theoretically, if UFBs promote the expansion of leaf area, the yield could not be improved in the canopies of fruit vegetables such as tomatoes (Higashide, 2013, 2015). To verify the effect
of UFBs on yield in fruit vegetables such as tomatoes and eggplants, analysis of yield-related elements is required. From our results, we could not find any elements related to yield improvement in UFB-treated tomatoes.

By using the model created in this study, it was possible to predict the effect of UFBs on the aboveground dry weight and LAI of tomato seedlings, although most of the predicted values were lower than the observed values (Fig. 2). These results showed the same tendency as reported by Higashide et al. (2014) and were particularly larger with a 16-h day length. With an 18-h day length, DM production is maximal, i.e., \( \frac{dM}{dt} = l(1 - e^{-\frac{dM}{p/d_t}}) \) (Table 2). On the other hand, with a 16-h day length, DM production is not maximal, i.e., \( \frac{dM}{dt} = \frac{dM}{p/d_t} \) (Table 5). The regression lines for the predicted values (y) against the observed values (x) were \( y = 0.94x \) for dry weight and \( y = 0.86x \) for LAI, indicating that the predicted values were lower than the observed values. This could imply changes in rates of leaf enlargement, and of DM production per leaf area. To further improve the prediction accuracy, it is necessary to further investigate the rates of leaf expansion and DM production and the increase in initial LMA should be considered.

We conclude that UFB treatment increased the leaf area expansion of tomato seedlings. Although this effect, i.e. promoting leaf expansion, was not be affected by assimilation conditions, DM production was affected. By using the model described here, this UFB treatment effect could be predicted with high accuracy.

**Literature Cited**

Fan, X., Z. Xu, X. Liu, C. Tang, L. Wang and X. Han. 2013. Effects of light intensity on the growth and leaf development of young tomato plants grown under a combination of red and blue light. Sci. Hortic. 153: 50–55.

Faria, T., D. Wilkins, R. T. Besford, M. Vaz, J. S. Pereira and M. M. Chaves. 1996. Growth at elevated CO₂ leads to down-regulation of photosynthesis and altered response to high temperature in Quercus suber L. seedlings. J. Exp. Bot. 47: 1755–1761.

Higashide, T. 2013. Greenhouse Tomato Yield and Solar radiation. In: T. Higashide (ed.). Tomatoes: Cultivation, Varieties and Nutrition. p. 3–18. Nova Science Publishers, New York.

Higashide, T. 2015. Factors pertaining to dry matter production in tomato plants. In: T. Higashide (ed.). *Solanum Lycopersicum*: Production, Biochemistry and Health Benefits. p. 1–23. Nova Science Publishers, New York.

Higashide, T. and E. Heuvelink. 2009. Physiological and morphological changes over the past 50 years in yield components in tomato. J. Amer. Soc. Hort. Sci. 134: 460–465.

Higashide, T., M. Narukawa, Y. Shimada and K. Soeno. 2014. Suppression of elongation and growth of tomato seedlings by auxin biosynthesis inhibitors and modeling of the growth and environmental response. Sci. Rep. 4: 4556. DOI: 10.1038/srep04556.

Himuro, S. 2016. Biological applications of fine bubbles. Jpn. J. Multiphase Flow 30: 10–18 (In Japanese with English abstract).

Kido, T. 2016. The possibilities of the fine bubble. Food Processing and Ingredients 49: 20–21 (In Japanese).

Minagawa, H., K. Fujiwara, R. Kurimoto, T. Yasuda, E. Harada and N. Hata. 2016. Effects of microbubbles on germination and growth of spinach. J. JSEM 16: 77–83 (In Japanese with English abstract).

Nakano, A., N. Hara, K. Mizuno and T. Ikebe. 2010. Effect of CO₂ and O₂ enrichments on rose growth. Agriculture and Horticulture 85: 910–915 (In Japanese).

Nakano, Y. 2016. Fine bubble electrolyzed water for hygiene management. Food Processing and Ingredients 49: 29–32 (In Japanese).

Nanya, K., Y. Ishigami, S. Hikosaka and E. Goto. 2015. Effects of far-red LED light on the growth and development of tomato seedlings in a closed seedling production system. J. SHITA 27: 61–67 (In Japanese with English abstract).

Nishihara, I. and S. Maeda. 2014. Ultrafine bubbles-generation, measurement, and application in the fields of food, agriculture, and cleaning. Food Processing and Ingredients 49: 26–29 (In Japanese).

Paul, M. J. and T. K. Pellny. 2003. Carbon metabolite feedback regulation of leaf photosynthesis and development. J. Exp. Bot. 54: 539–547.

Yamamoto, A., H. Nishikawa, N. Sakurai, S. Maeda, I. Nishihara and T. Hayashi. 2016. Ultrafine bubbles enhance growth of *Solanum lycopersicum* young plants. Hort. Res. (Japan) 15 (Suppl. 2): 160 (In Japanese).

Yamamoto, A., H. Nishikawa, N. Sakurai, S. Maeda, I. Nishihara and T. Hayashi. 2017. Sequential events caused by ultrafine bubbles on *Solanum lycopersicum* roots. Hort. Res. (Japan) 16 (Suppl. 1): 101 (In Japanese).