Dry-fog Aeroponics Affects the Root Growth of Leaf Lettuce (Lactuca sativa L. cv. Greenspan) by Changing the Flow Rate of Spray Fertigation

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The growth characteristics and physiological activities of leaves and roots of lettuce cultivated in dry-fog aeroponics with different flow rates of nutrient dry-fog (FL, 1.0 m s⁻¹; NF, 0.1 m s⁻¹) were investigated under a controlled environment for two weeks and compared to lettuce cultivated using deep-flow technique (DFT). The growth of leaves of FL and DFT was not different and was significantly higher than that of NF. The amount of dry-fog particles adhering to the objects was higher in FL than in NF, so that the root growth in NF was significantly higher than that of FL. The respiration rate of roots was significantly higher in dry-fog aeroponics, but the dehydrogenase activity in the roots was significantly higher in DFT. There were no differences in the contents of chlorophyll and total soluble protein in the leaves or the specific leaf area. Photosynthetic rate and stomatal conductance were higher in dry-fog aeroponics. The contents of nitrate nitrogen, phosphate and potassium ions in the leaves were significantly higher in DFT, but the content of calcium ions was significantly higher in FL. Thus, changing the flow rate of the dry-fog in the rhizosphere can affect the growth and physiological activities of leaves and roots.

Keywords: hydroponics, photosynthesis, root hair, root respiration, soilless culture, stomatal conductance

INTRODUCTION

Dry-fog aeroponics is a new soil-less hydroponic technique that fills the rhizosphere with an extremely fine fog of atomized liquid fertilizer using a specialized nozzle as a double-fluid atomizer with nutrient solution and compressed air (Hikosaka et al., 2014). In dry-fog aeroponics, the fog in a chamber made of lightweight polystyrene is less than 10 μm in diameter on average per droplet, so less nutrient solution exists in the hydroponic system than in the general aeroponics system that is used for research and commercial cultivation (Biddinger et al., 1998; Farren and Mingo-Castel, 2006; He et al., 2013). Dry-fog nutrients that are not absorbed by the roots condense and fall down the inner wall to accumulate at the bottom of the chamber, and the collected solution is atomized again. Thus, the nutrient solution is circulated in the system, and there is no wastewater outside of the system. Because of these features, dry-fog aeroponics is expected to be an effective technique for protected cultivation to address the food shortage in the future caused by decreases in both arable land and water resources and cultural eutrophication caused by nutrient wastewater. Spray culture using a fog box with nozzles as double-fluid atomizers (Ehara et al., 1966) or with an ultrasonic humidifier, which can atomize the very fine fog, has been used for aeroponic systems, and many bioreactors have been developed to generate nutrient fog (Mohammad et al., 2000; Chun-Zhao et al., 2003), but the advantage of these methods for practical cultivation is not well understood.

The cultural feature of dry-fog aeroponics is the aerobic environment in the rhizosphere. In hydroponics, the dissolved oxygen level in the nutrient solution affects the respiration rate of roots and growth, which increase under aerobic conditions (Changhoo and Takakura, 1994; Yoshida et al., 1997). In dry-fog aeroponics, plant roots are hanged in the foggy nutrient solution and absorb water and nutrients directly from the fog particles. No part of the roots is soaked in the nutrient solution, and fresh airflow is always supplied to the root surface. Because the flow rate of dry-fog sprayed by a nozzle can be changed easily using a fan set in a chamber, it is possible to control the density and speed of the particles of nutrients and water adhering to the roots.

The growth promotion and increased viability of roots in an aerobic rhizosphere filled with foggy nutrient solution and an increase in the water absorption efficiency by continuous flow of dry-fog on the surface of roots are expected in dry-fog aeroponics. There has been no report indicating a relationship between the flow rate of foggy nutrient solution sprayed in the rhizosphere and plant growth with aeroponics, therefore, we cultivated lettuce plants with the dry-fog aeroponic technique and investigated the effect of the flow rate by changing it with a controlled fan set in the rhizosphere on the growth characteristics and physiological activities of plants.
MATERIALS AND METHODS

Dry-fog aeroponic system

Two dry-fog aeroponic systems (Hikosaka et al., 2014) consisting of a rhizosphere chamber made of polystyrene (1000 × 660 × 300 mm) and a dry-fog atomizing nozzle (developed and patented by Ikeuchi Co., Ltd., Osaka, Japan) were settled in a temperature-controlled room. Each chamber had 22 holes (20 mm across) for setting plants on the top, and only the roots remained in the chamber. An axial flow fan was equipped in one chamber, and the dry-fog flow rate was adjusted to 1.0 m s⁻¹ (FL). The other chamber was not equipped with a fan, and the flow of the dry-fog did not occur in the chamber except for the spraying from the nozzle (NF). The flow rate in the chamber was measured at a depth position of 70 mm from the planting hole using an omnidirectional anemometer (testo445; TESTO, Lenzkirch, Germany) (Fig. 1). The nozzle continuously atomized fine foggy nutrients of a commercial liquid fertilizer (OAT Agrio Co., Ltd., Tokyo, Japan, EC 1.2 mS cm⁻¹, pH 6.0, N: 130 ppm, P: 60 ppm, K: 200 ppm, Ca: 115 ppm, Mg: 30 ppm, Fe: 1.4 ppm, Mn: 0.8 ppm, Zn: 0.05 ppm, Cu: 0.02 ppm, Mo: 0.02 ppm) that always filled the chamber. As a control experiment, a deep-flow technique (DFT) hydroponic chamber (480 × 380 × 114 mm) was filled with 30 L of the same liquid fertilizer. To confirm the amount of dry-fog particles flowing through the rhizosphere, a slide glass that was painted with thin silicon oil (1000 cs) was exposed perpendicularly upstream of the dry-fog for one second. Then, the number and size of particles that were trapped in the silicon oil layer on a slide glass were observed and recorded with a microscope (Fig. 1, Photos).

Plants and culture conditions

Commercial leaf lettuce seeds (Lactuca sativa L. cv. ‘Greenspan’, Kaneko Seeds Co., Ltd., Tokyo, Japan) were sown on sponge blocks (20 × 20 × 30 mm) and were supplied with enough pure water. The air temperature and relative humidity in the cultivation room were maintained at 25°C and 60%, respectively. The photosynthetic photon flux density (PPFD) was 120 μmol m⁻² s⁻¹ supplied by red (660 ± 10 nm, 96 μmol m⁻² s⁻¹) and blue (455 ± 10 nm, 24 μmol m⁻² s⁻¹) light-emitting diodes (LEDs) lamps (Legu LED, HRD Co., Ltd., Tootori, Japan) with a 16-h day length. After germination, half-strength liquid fertilizer (EC 0.6 mS cm⁻¹, pH 6.0) was supplied by bottom irrigation for two weeks, and 20 seedlings that grew uniformly were transplanted in each hydroponics system and were grown for two weeks.

Measurements of growth and photosynthesis

Every week after transplanting, six plants were sampled from each hydroponic system, and the fresh weight, the number of leaves and the fresh weight of roots were recorded. On the 2nd week after transplantation, a leaf disk (10 cm²) was sampled from each plant and dried in an oven at 80°C for two days and its dry weight was measured to estimate the specific leaf area. Transpiration and the CO₂ assimilation rates of a fully expanded mature single attached leaf were measured under controlled environmental conditions using an LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA) on the 2nd week after transplanting. The measurement conditions were as follows: leaf temperature, 25°C; PPFD supplied by a red-blue LED light source (10% blue based on PPFD), 150 μmol m⁻² s⁻¹; leaf vapor pressure deficit, 1.0 kPa; and ambient CO₂ concentration, 500 μmol mol⁻¹.

Measurements of root activity

On the 2nd week after transplanting, intact root sam-

Fig. 1 Flow rate (m s⁻¹) in the rhizosphere at each planting hole (filled circles in A, FL and in B, NF) and cutaway drawing of the dry-fog aeroponic chamber (C). Photographs show the droplets of dry-fog trapped in the thin silicon oil layer on a slide glass exposed perpendicularly upstream of the dry-fog in the rhizosphere (dotted line position) in FL (A) and NF (B). Dotted line shows the position of slide glass in the chamber (A, B: horizontal; C: vertical).
samples were taken from six growing plants that developed new roots under each hydroponic condition, and the rates of root respiration were measured polarographically at 25°C using a Clark-type gas-phase oxygen electrode (CB1D; Hansatech, Norfolk, UK) with incoming humidified ambient air (21% O₂) under dark conditions. After measuring the respiration rate, the root was blotted by pressing slightly for 10 s between two sheets of filter paper (No. 1, ADVANTEC, Tokyo, Japan), then fresh weight was measured and the dehydrogenase activity of the roots was tested according to the triphenyltetrazolium chloride (TTC) method. Root samples (1.0–1.5 g FW) were cut into small pieces of 10 mm and placed in a tube containing 5 ml of equally mixed solution of 0.1% TTC and 0.1 M phosphate buffer (pH 7.0) for a reduction reaction for 2 h at 37°C. Then, 2 ml of 1 M H₂SO₄ was added to the tube to stop the reaction. The root was blotted with paper towel and homogenized in ethyl acetate. The extract was transferred to a tube and was quantified to 6 ml by adding ethyl acetate. The absorbance of the extract at 485 nm was recorded using a spectrophotometer, and the concentration of triphenylformazan (TPF) as a reaction product was calculated by comparing to a standard curve with diluted TPF after complete reduction of 0.1% TTC (Wako Pure Chemical Industries, Ltd., Osaka, Japan) with Na₂S₂O₃.

Measurement of leaf constituents

The contents of nitrate nitrogen, calcium ions, phosphate ions and potassium ions in the leaves were measured every week, and the total soluble protein (TSP) and chlorophyll were measured on the 2nd week after transplantation. The leaves (3 g FW) were homogenized in deionized water. The homogenate solution was passed through a filter paper (No. 1, ADVANTEC, Tokyo, Japan) and was measured using RQflex (Merck Millipore, Darmstadt, Germany). Because RQflex has previously used for measuring the ion contents (Kintzios et al., 2004) and it was reported that the measurements were closely correlated with HPLC analysis (Ito et al., 2013). In present study, accurate quantitative correction was not carried out because of comparative experiments. Other leaves (2 g FW), which were sampled for measuring the concentration of TSP, were homogenized in 50 mM Tris-HCl buffer (pH 8.0) containing 10 mM MgCl₂, 0.2 mM ethylenediamine tetra-acetic acid and 5 mM dithiothreitol on ice, and 1 ml of the extract was transferred to a tube. Then, 1 ml of 20% (w/v) trichloroacetic acid was added to the tube and left for more than 15 min. The supernatant was removed, and the pellet was resolved in 1 ml of 1 M NaOH for 24 h. The TSP concentration of the samples was measured by the Coomassie Brilliant Blue protein assay (Nacalai tesque, Kyoto, Japan) and calculated by comparing to a standard curve with diluted BSA (Nacalai tesque, Kyoto, Japan). The chlorophyll content of the leaves (1 g FW) was analyzed after extraction by grinding the leaves in pre-cooled 80% (v/v) acetone and quantified spectrophotometrically according to the method of Arnon (1949).

Data analysis

All of data were subjected to a one-way analysis of variance (ANOVA) and the mean differences were compared using the Tukey’s HSD test when the F-test indicated a significant difference at P ≤ 0.01. The hydroponic culture experiment was repeated twice under the same conditions; each data point was the mean of 12 replicates, and a comparison with P ≤ 0.05 was considered significantly different.

RESULTS AND DISCUSSION

Flux density of dry-fog particles and lettuce growth

The number of dry-fog particles trapped in the silicone oil layer on a slide glass was apparently superior in FL than in NF (Fig. 1 Photos), meaning that the rhizosphere with fast flow rate was filled with much of foggy water and nutrient solution compared to that with low flow rate. In FL, approximately three times the nutrient droplets were collected on the silicone oil layer compared to that in NF (Table 1). There are direct observation showing a relationship between the flow rate of dry-fog spray and the amount of dry-fog particles in the root zone. The average diameter of the droplets was less than 10 μm in both FL and NF and showed no significant difference. There was no difference other than in the flow rate for the supply of nutrient solution. Because a nozzle continuously sprayed dry-fog and saturated relative humidity was maintained in a chamber filled with dry-fog nutrients, more foggy nutrient droplets can contact the root surface under active flow conditions than under non-flow conditions. So, it was thought that the roots in FL could absorb water and nutrients easier than in NF when the roots had same volume and same surface area.

The growth of leaves at each week after transplantation was significantly higher in FL than in NF but showed no difference between FL and DFT (Fig. 2). The growth of roots on the 1st week in both dry-fog cultures was significantly higher than that in DFT but showed no difference between the two dry-fog cultures. The root fresh weight in NF on the 2nd week was significantly higher than those in other two by more than double of DFT. As a result, the top/root fresh weight ratios (T/R) in dry-fog aeroponics were significantly lower at each week compared to that of DFT. We already reported in dry-fog aeroponics with altering fog particle size that the T/R of lettuce decreased between the two dry-fog cultures. The root fresh weight in NF on the 2nd week was significantly higher than those in other two by more than double of DFT. As a result, the top/root fresh weight ratios (T/R) in dry-fog aeroponics were significantly lower at each week compared to that of DFT. We already reported in dry-fog aeroponics with altering fog particle size that the T/R of lettuce decreased with increased root growth compared to shoot growth in the

| Table 1 | Average diameter and density of droplets trapped in the silicone oil layer. |
|---------|---------------------------------------------------------------|
|         | Average number of droplets (mm⁻³) | Distribution of the droplet diameter (%) | Average diameter of droplets (μm) |
|         | ~10 μm | 10~20 μm | 20~30 μm | 30 μm~ |         | 10 μm | 20 μm | 30 μm | 40 μm |        |
| FL      | 1905±179 | 15.1 | 83.0 | 1.4 | 0.1 | 9.5±0.25 |
| NF      | 867±143  | 15.1 | 9.0  | 0.5 | 0.4 | 9.2±0.43 |

Means ± standard deviations.
We hypothesized that, in the dry-fog culture, lettuce roots in the aerobic rhizosphere absorbed enough water and nutrients from the foggy particles flowing in the root zone by altering root morphogenesis resulted from developing branched roots with root hairs and by enlarging root biomass resulted from distributing new assimilates preferentially into the growth of roots rather than leaves, and these growth and developmental changes are adaptations to the aerobic rhizosphere environment by increasing root surface area to improve the efficiency of catching dry-fog particles and absorbing water and nutrients. We considered that the roots that grew in FL quickly adapted to aerobic rhizosphere conditions within one week after transplantation and that shoot growth increased in the 2nd week, but the roots that grew in NF could not completely adapt within one week and maintained assimilation partitioning to increase root growth in the 2nd week. Although root growth had priority over shoot growth for catching dry-fog nutrients efficiently, the shoot fresh weight showed no significant difference in either week after transplantation between FL and DFT, whose roots grew without any morphological changes. It is expected that well-developed roots in dry-fog aeroponics promote the absorption of water and nutrients and subsequent shoot growth. Therefore, we concluded that dry-fog aeroponics increases shoot growth after the adaptation of roots to the aerobic conditions in the rhizosphere, and further investigations are needed for the growth characteristics after three weeks.

**Physiological activity of the leaves and roots**

The photosynthetic rate of the attached mature leaves in the 2nd week after transplantation increased significantly by dry-fog aeroponics and was significantly higher in NF than FL (Table 2). The stomatal conductance was also higher in dry-fog aeroponics, especially in NF, whose leaves absorbed more CO$_2$ into the intercellular space than did those that were grown in DFT under the same atmospheric CO$_2$ concentration. There were no differences in the contents of chlorophyll and TSP and SLA, indicating the thickness of a leaf, among all of the hydroponic systems, so it was thought that the photosynthetic rate of leaves grown under dry-fog aeroponics increased due to the elevated CO$_2$ concentration in leaves with opening stomata and not due to an increased photosynthetic ability.

In terms of the root morphogenesis in sprayponics, it is particularly worth noting that root hairs formed on the surface of roots under the dry-fog aerobic conditions because of no related reports found so far. Bibikova and Gilroy (2003) reported that the development of the root hair was influenced by abscisic acid (ABA) production under water stress. Furthermore, the availability of phosphorus or nitrate in the rhizosphere affects the length, number and density of root hair (Bates and Lynch, 1996; Ma et al., 2001; Hammac et al., 2011). Thus, the development of root hair in some kinds of plant species may be related to water stress or nutrient stress as a survival adaptation under the unhealthy rhizosphere environment. However, in this study, lettuce plants had never experienced both water and nutrient shortages because of the continuous spraying with nutrient solution at proper concentration all the day. So the development of root hair in dry-fog aeroponics was not affected by the water stress related ABA biosynthesis, because the stomatal conductance of dry-fog aeroponics, especially in NF with significantly developed root hair, was higher than DFT where plants were never exposed to water stress. This increased stomatal conductance at the 2nd week after transplanting to dry-fog aeroponics showed that the evapotranspiration demand was filled much more in dry-fog aeroponics than in DFT by the enough absorption of moisture.
of water resulted from increased surface area of roots and decreased T/R.

The respiration rate of roots is an important physiological parameter for evaluating root activity for absorbing water and nutrients. There was no significant difference in the root respiration rate within dry-fog aeroponics (FL vs. NF) and that of the DFT was significantly lower compared to those of dry-fog aeroponics in the 2nd week after transplantation (Table 2). These results indicate that the roots increased not only the surface area of developing root hairs to improve the dry-fog trapping efficiency but also the root absorption activity of water and nutrients adhering to the root surface.

On the other hand, the TTC reducing activity was significantly higher in DFT, but there was no difference between FL and NF (Table 2). TTC is absorbed into tissue cells and reduced to red-colored TPF, which has been used as an indicator of dehydrogenase activity in many studies of root viability. At the same time, TTC reduction activity is also used as a parameter to evaluate uptake of water and nutrients (Wang et al., 2006). It has been hypothesized that good growth of shoots needs both a high TTC reduction activity and a large root surface area (Wen-Zeng et al., 2011). The TTC reduction activities of the roots in FL and NF were approximately 60% and 70% of those in DFT (Table 2), but the root fresh weights were 144% and 233% of those in DFT in the 2nd week after transplantation (Fig. 2). Considering total root activity as a product of TTC reduction activities per FW and total FW of whole roots per plant, there was no marked difference in the total absorption activity per plant between DFT and FL, and NF was expected to have the highest absorbing activity. Because the TTC absorbed by roots is reduced mainly by dehydrogenase in mitochondria, the relationship between the TTC reduction activity and respiration rate of roots has been investigated (Louise et al., 2000). Furthermore, considering that the root morphology changed drastically in dry-fog aeroponics, the physiological activities of roots are expected to adapt to the dry-fog rhizosphere environment. Increases in both stomatal conductance and photosynthetic rate of the plants grown in dry-fog rhizosphere might be caused by improving root morphology and physiological activity to enhance absorption of water and nutrient solution. These were results of root growth adaptation to the
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dry-fog environment at two weeks after transplanting, however, their influences to the leaf growth could be reflected in a short while later. Additional researches are necessary to explain the changes in root growth adaptation and growth of plants cultivated in dry-fog aeroponics for a longer period.

Leaf constituents

The contents of nitrate nitrogen and phosphate ions of mature leaves were the highest in DFT in both weeks (Fig. 4). FL had significantly higher values than those of NF in the 1st week, but there was no significant difference between these values in the 2nd week due to the increased surface area of the roots in NF. Because the roots in NF had almost the same absorption activity of nutrients to FL, increases in both the biomass and the absorbing activity of the roots could promote the shoot growth after the 3rd week after transplanting. Although the roots in dry-fog aeroponics were less exposed to nutrient solution in the rhizosphere than in DFT and the leaf contents of nitrate nitrogen and phosphate were lower in both FL and NF than those in DFT with unchanged chlorophyll content, no nutrient deficiencies were observed in the leaves in dry-fog aeroponics. The content of potassium ions in mature leaves was also significantly higher in DFT in both weeks compared to that in dry-fog aeroponics. NF had a significantly higher value than FL in the 2nd week. The content showed no significant changes between the 1st and 2nd weeks in DFT and FL, but NF increased this content by 30%. The content of calcium ions in mature leaves significantly increased in the FL than DFT in both weeks, and the FL was higher than the NF in the 2nd week. Because calcium and potassium have a competitive relationship, enhancing potassium absorption in NF in the 2nd week inhibited an increase in calcium. There was no significant difference in the calcium content between NF and DFT in the 2nd week. The insufficient absorption and transportation of calcium causes the physiological disorder “tip-burn” in the shoots of lettuce (Bangerth, 1979; Barta and Tibbitts, 2000) and decreases the production of lettuce. Many studies have investigated the improvement of calcium absorption during the cultivation of leafy vegetables (Shibata et al., 1995; Goto and Takakura, 2003; Takahashi et al., 2012). Although tip-burn did not occur in lettuce cultivation with dry-fog aeroponics, lettuce plants can grow well with healthy consumption values. We suggest that dry-fog aeroponics inhibits the occurrence of tip-burn in lettuce production with minimum amounts of nutrient solution and water.

CONCLUSION

In the dry-fog aeroponics, the flow rate of dry-fog through the rhizosphere can be controlled easily by using a fan. The shoot and root growth of leaf lettuce plants was altered depend on the flow rate, which affected on the amount of dry-fog particles that adhere to the surface of the roots. The roots grew and developed adaptively to dry-fog aerobic rhizosphere with well branching, root hairs and high respiration activity. These roots might be prefer to increase ability of water and nutrient absorption, lead to fill the evapotranspiration demand sufficiently and enhance the photosynthetic rate and stomatal conductance. Accordingly, dry-fog aeroponics is thought to be an effective cultivation system to promote plant growth.

As the flow rate of dry-fog aeroponics indirectly affects on the plant growth by the root development, optimally-controlled flow rate might make it possible to accelerate the root growth adaptation, thereby leading to improved growth in the early stages after planting. Therefore, further investigations are necessary to better understand the optimal rhizosphere environment for root growth that makes the production yield maximum in dry-fog aeroponics.
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