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A novel aeroponic technique using dry-fog spray fertigation to grow leaf lettuce (*Lactuca sativa* L. var. *crispa*) with water-saving hydroponics

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Abstract: Growth characteristics of lettuce cultivated using a novel "dry-fog" hydroponic technique were investigated and compared to lettuce cultivated using deep flow technique (DFT) as the prevailing hydroponic technique. Dry-fog hydroponics is an aeroponic technique that sprays a very fine foggy nutrient solution with an average droplet diameter of less than 10 µm into the root zone. The roots extend into the chamber filled with dry-fog of the liquid fertilizer and absorb water and nutrients from the dry-fog that fills the rhizosphere. This soilless culture system needs less water than any other hydroponic technique, and no differences were found in growth and harvest quality of plants between the two tested sytems. For dry-fog culture, root growth was encouraged and root hair significantly developed to catch the foggy nutrient solution efficiently. The contents of ascorbic acid, nitrate nitrogen, Ca^{+} and chlorophyll of leaves were not significantly different between the two hydroponic cultures. However, respiration rate of roots and photosynthetic rate of leaves significantly increased with dry-fog culture. Because the amount of water around the roots is less with dry-fog, horticultural crops are expected to grow well with this novel hydroponic technique which optimizes the growth and quality of plants with water-saving hydroponics.

1. Introduction

To address the food shortages in the near future caused by a decrease in arable land or increase in the world population, greenhouse horticultures, especially hydroponic cultures without soil, are becoming more important. While one advantage of a hydroponic culture is that plants can be cultivated under optimally controlled conditions for nutrients regardless of the soil conditions, a large amount of water is required to grow crops with nutrient solution. New hydroponic systems that enable stable crop production while saving water have been anticipated in recent years. Aeroponics is a water-saving hydroponic technique without rooting media (Weathers and Zobel, 1992; Ritter *et al*., 2001; Farren and Mingo-Castel, 2006). In this culture, liquid fertilizer is periodically sprayed on the roots from nozzles with pipes in the rooting zone. The sprayed nutrient solution that is not absorbed by the roots is usually re-sprayed using a re-circulation system or it is discarded directly. Tomato, one of the most important crops world-wide, has recently been cultivated using aeroponics (Biddinger *et al*., 1998; Zhao *et al*., 2010). One of the features of this culture system is nutrient accessibility in which nutrients are absorbed efficiently by the roots. Furthermore, root growth in most vegetable crops is expected to increase under the aerobic conditions of the rhizosphere environment because there is no solid phase and less liquid phase compared to the physical composition of soil or any other rooting media (Hillel and David, 1988; Cherif *et al*., 1997).

Dry-fog aeroponics is a novel hydroponic technique that sprays a very fine fog of atomized liquid fertilizer using a specialized nozzle as a double-fluid atomizer with nutrient solution and compressed air (developed and patented by Ikeuchi Co., Ltd., Osaka, Japan). Dry-fog is the finest fog and is less than 10 µm in diameter on average per droplet. The droplets are too small to wet objects, such as roots. Currently, dry-fog is mainly used to humidify factories producing electronic components or printing to keep the environment static-free and has not yet been applied to crop cultivation in commercial horticulture. In dry-fog aeroponics, dry-fog fertilizer is atomized using a specialized nozzle in the rhizosphere where the plant roots directly absorb water and nutrients from the dry-fog (Fig. 1), like an aerial root of epiphytic orchids. Because the fine, foggy, nutrient solution is atomized, only one specialized nozzle can occupy the large space for the rooting zone in a chamber (1000×660×300 mm), with a smaller amount of water compared to the other types of predominant spray hydroponic techniques; therefore, dry-fog aeroponics can reduce water usage and the number of nozzles that are needed, resulting in a lower running cost and greater environmental conservation. The dry-fog nutrients that are not being absorbed by the roots accumulate as condensation on the inner wall of the cultivation chamber, and the collected condensation in the nutrient reservoir is atomized again. Thus, there

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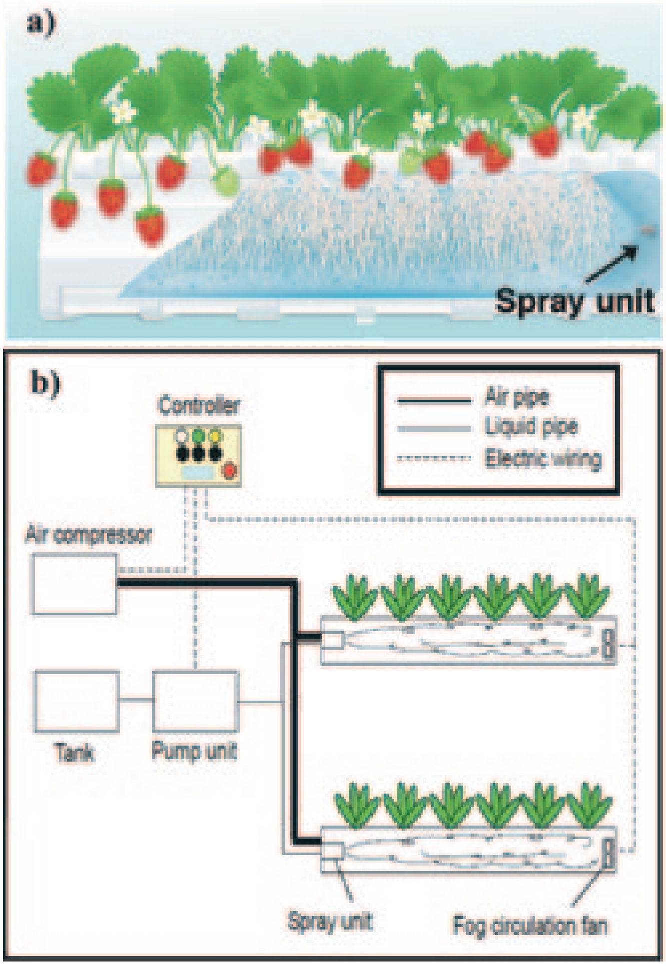

Fig. 1 - a) Cutaway drawing of the dry-fog hydroponic chamber. b) System chart of the dry-fog hydroponic technique.

is little puddling of the fertilizer solution in the chamber, and the roots hang in the dry fog. An ultrasonic humidifier also can atomize the very fine fog, and has been used for many bioreactors to generate the nutrient fog (Mohammad *et al.*, 2000; Lin *et al.*, 2003; Jing and Yunwei, 2013). In contrast, it is believed that the nozzle used for dry-fog aeroponics atomizes a larger amount of fine fog at a low cost and without problems. Dry-fog aeroponics has not yet been investigated or established as a commercial cultivation system for horticultural crop production.

In the present study, leaf lettuces were cultivated using dry-fog aeroponics in a controlled environment. The growth and leaf constituents were measured and compared to plants cultivated with traditional hydroponic techniques (Deep Flow Technique) to evaluate the efficacy of dry-fog aeroponics. Root respiration and leaf photosynthetic rate were also investigated and compared in order to evaluate affects on water and nutrient absorption resulting from differences in the rhizosphere environment in the two hydroponic systems.

2. Materials and Methods

A plastic chamber (1000×660×300 mm) was fitted with a dry-fog atomizing nozzle to establish a dry-fog cultivation system (Fig. 1). A double-fluid atomizer nozzle was connected to the nutrient solution reservoir with a plastic tube (6 mm across) and a compressor with Teflon tube (8 mm across), with an air pressure of 0.3 MPa. Size fractionation of atomized droplets was measured using LDSA-SPR (Tohnichi computer applications Co. Ltd., Tokyo, Japan), which analyzes the droplets by Fraunhofer diffraction and calculates the size distribution of the droplets by Rosin-Rammler distribution. A commercial liquid fertilizer (Otsuka Chemical 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, B: 0.75 ppm, Mo: 0.02 ppm) was added to the nozzle from a nutrient solution reservoir equipped at the end of an aeroponic chamber. The nozzle atomized fine foggy nutrients that continuously filled the chamber. Each chamber had 48 holes (20 mm across) across the top to hold plants and only the roots remained in the chamber. As a control experiment, a Deep Flow Technique (DFT) hydroponic chamber (800×665×310 mm) was filled with 30 L of the same liquid fertilizer. Leaf lettuce (*Lactuca sativa* L. cv. Okayama Saradana, Takii Co. Ltd., Kyoto, Japan) was sown on sponge blocks (10×10×20 mm) supplied with enough pure water. The air temperature and relative humidity throughout the cultivation were maintained at 26°C and 60% respectively. The photosynthetic photon flux density was 250 µmol m^{-2} s^{-1} supplied by cool white tubular fluorescent lamps with a 16-h day length. After germination, the liquid fertilizer (EC 0.6 mS cm^{-1}, pH 6.0) was supplied by bottom irrigation for two weeks, and 48 seedlings that had grown uniformly were transplanted into each hydroponic system and grown for four weeks. Every week, plants were sampled from both hydroponic systems and the fresh weight, leaf length, and fresh weight of roots were recorded. After sampling, the leaves and roots were dried in an oven at 80°C for two days and the dry weights were recorded. At three and four weeks after transplantation, intact root samples (1 g FW) were taken from a growing plant that developed new roots under hydroponic conditions, and the rate of root respiration was measured polarographically at 26°C using a Clark-type gas-phase oxygen electrode (CB1D, Hansatech, Norfolk, UK) with incoming humidified air and 21% O_2 under dark conditions.

The contents of nitrate nitrogen, calcium ion, ascorbic acid and chlorophyll in the leaves were measured at three and four weeks. Leaves (10 g FW) were sampled to determine nitrate nitrogen and calcium contents. The leaves were homogenized in de-ionized water and the nitrate nitrogen and calcium contents in the filtrated fraction were measured using RQ-FLEX (Merck Millipore, Darmstadt, Germany). The other leaves (10 g FW) were also sampled and homogenized in a 5% metaphosphoric acid solution, and the content of ascorbic acid in the filtrated fraction was measured using RQ-FLEX. The content of leaf chlorophyll was measured using SPAD meter (SPAD-502, KONICA MINOLTA, INC., Tokyo, Japan). At four weeks after planting, CO_2 assimilation rates (A) of fully expanded mature leaves were measured under different CO_2 concentrations (50-1500 µmol mol^{-1}) using an LI-6400 portable photosynthesis system

(Li-Cor, Lincoln, NE, USA). The measurement conditions were leaf temperature, 20°C, and photosynthetic photon flux densities, 1200 μmol $m^{-2} s^{-1}$. The maximum rate (Max) and apparent CO_2 fixation efficiency (ϕ) were estimated as parameters of the best-fitted non-rectangular hyperbola for the photosynthetic responses to intercellular CO_2 concentrations (Ci). All data were subjected to one-way analysis of variance (ANOVA), and the mean differences were compared using the Tukey HSD test when the *F*-test indicated a significant difference at $P≤0.01$. Each data point was the mean of six replicates and a comparison with $P≤0.05$ was considered significantly different.

3. Results and Discussion

In order to characterize dry-fog spray fertigation conditions, the fog was measured using LDSA-SPR of droplets of various sizes (Fig. 2). The minimum and maximum diameter of droplets were 1.64 μm and 149 μm, respectively. LDSA-SPR analyzed the size distribution of droplets, and the Sauter mean diameter, which commonly means the average particle size of powders and liquid droplets, was found to be 8.85 μm by calculation. Therefore, the fog in the chamber was certainly dry-fog.

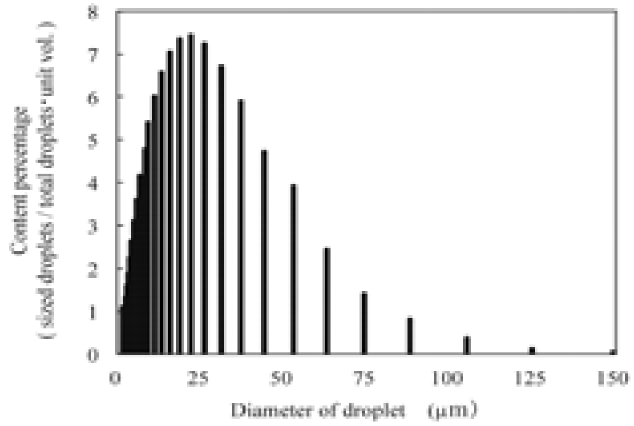

Fig. 2 - The particle size distribution of droplets using a double-fluid atomizer nozzle to establish a dry-fog hydroponic technique measured by LDSA-SPR. The mean diameter of droplets was analyzed and calculated based on this distribution.

There was no significant difference in the fresh and dry weight of leaves between the dry-fog and DFT hydroponic cultures (Fig. 3). Root growth was not significantly different at three weeks after planting, but during the fourth week of dry-fog culture the root fresh weight increased by 29% compared to roots grown using DFT. The roots from the dry-fog culture had many lateral roots, and many root hairs developed on the surface. This development was not observed in the roots grown using DFT (Fig. 4). The root respiration rate at four weeks after planting was significantly high in the dry-fog culture, but there was no significant difference at three weeks between the two hydroponic

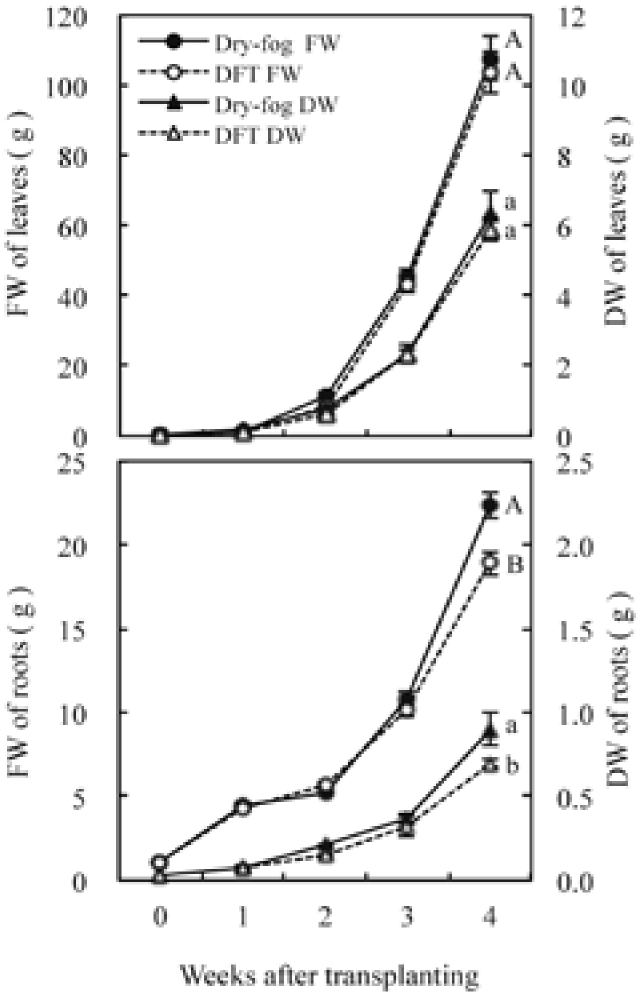

Fig. 3 - Changes in fresh and dry weights of lettuce leaves and roots grown using dry-fog or DFT hydroponics during the four weeks after transplanting. Different uppercase and lowercase letters show a significant difference ($P≤0.05$), bars represent ±SE (n=6).

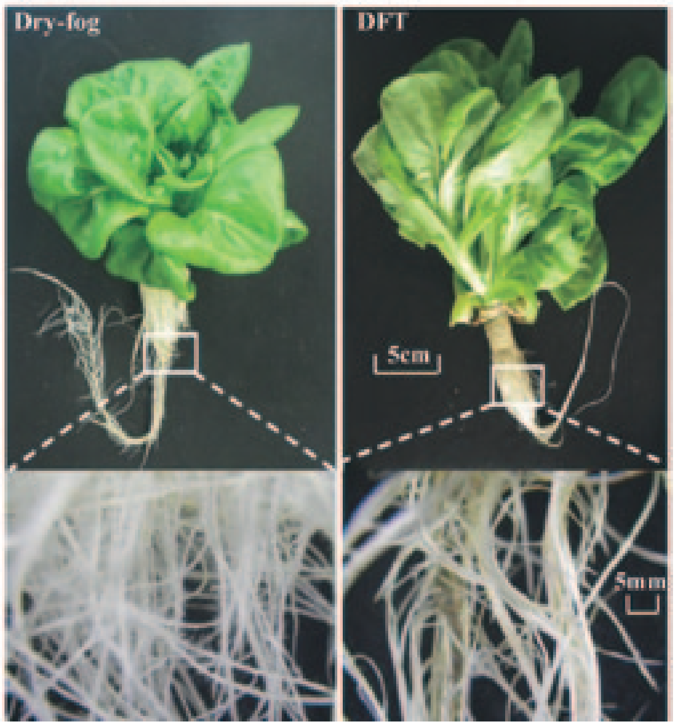

Fig. 4 - Leaves and roots of the lettuce grown using dry-fog or DFT hydroponics at four weeks after transplanting.

systems (Fig. 5). The photosynthetic rates of the mature leaves grown for four weeks dry-fog culture significantly increased in both limited and saturated Ci compared to those in DFT (Fig. 6). In both hydroponic cultures, the photosynthetic rate increased linearly up to approximately 300 μmol mol^{-1} Ci and saturated at approximately 600 μmol mol^{-1} Ci. Leaf nutrient contents were compared between the two hydroponic cultures at three and four weeks after transplanting (Fig. 7). The nitrate nitrogen content in the leaves grown using dry-fog culture significantly

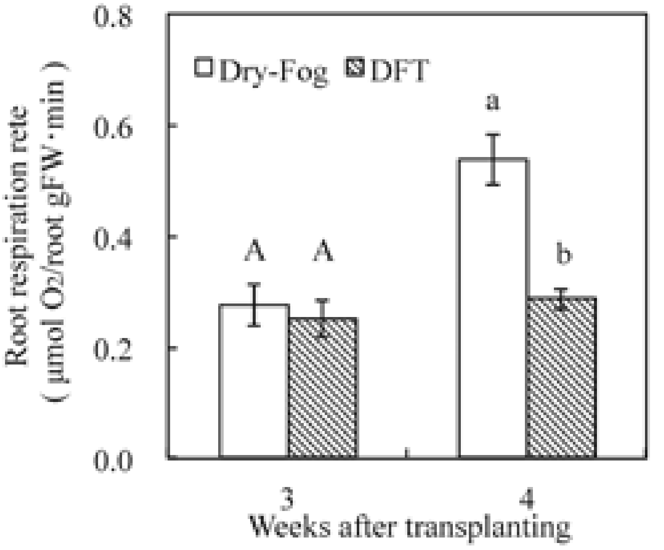

Fig. 5 - Respiration rates of lettuce roots grown using dry-fog or DFT hydroponics for three and four weeks after transplanting. Different letters show significant difference ($P \le 0.05$), bars represent ±SE (n=6).

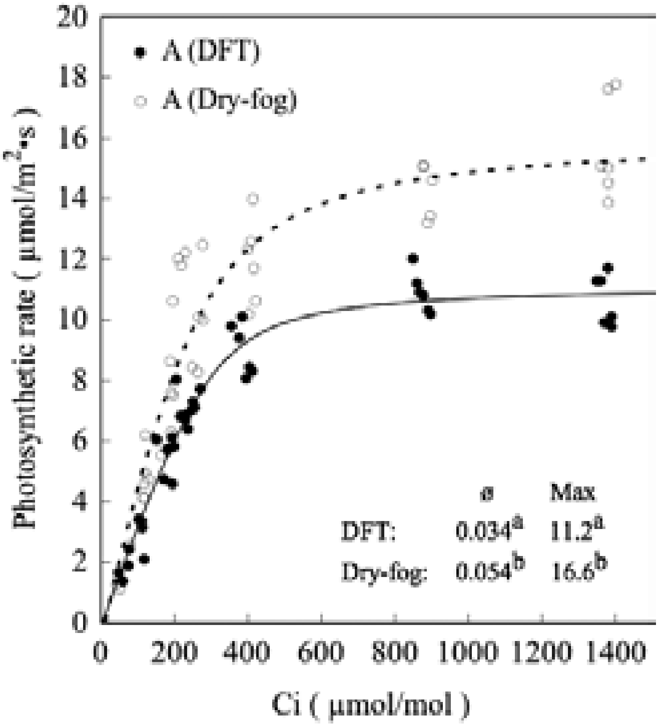

Fig. 6 - Relationships between CO_2 assimilation rates (A) and intercellular CO_2 concentration (Ci) measured for the attached mature leaves of lettuce grown using dry-fog or DFT hydroponics for four weeks after transplanting. The saturated rate (Max) and the apparent CO_2 fixation efficiency (ϕ) were estimated as parameters of the best-fitted non-rectangular hyperbola response curve to Ci. Different letters show significant difference ($P \le 0.05$).

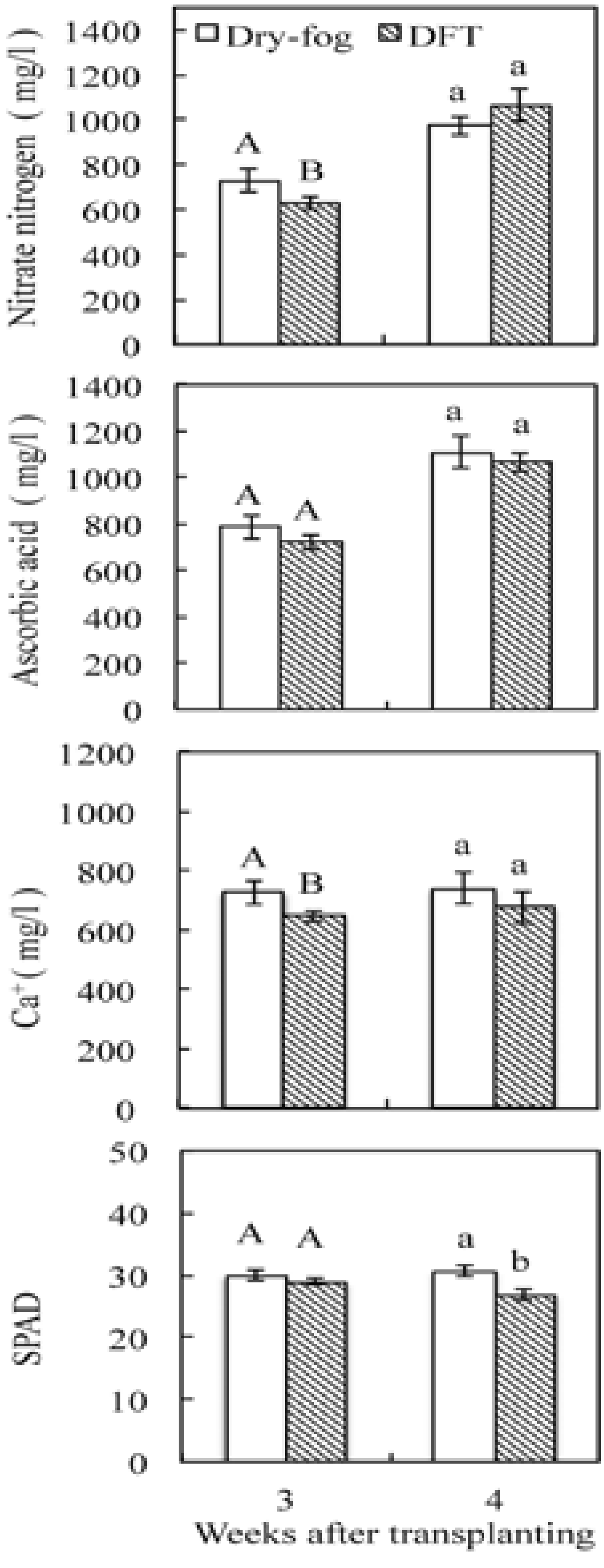

Fig. 7 - Nitrate nitrogen, ascorbic acid, calcium ion and chlorophyll content of mature lettuce leaves grown using dry-fog or DFT hydroponics for three and four weeks after transplanting. Different letters show significant difference ($P \le 0.05$), bars represent ±SE (n=6).

increased by 16% compared to those grown using DFT at three weeks after planting, but there was no significant difference at four weeks. The content of ascorbic acid in the leaves grown using dry-fog culture increased by 9% at three weeks and 4% at four weeks, compared to the leaves grown using DFT, however there were no significant differences. The calcium content in the leaves grown using dry-fog culture significantly increased at three weeks after planting, but there was no significant difference at four weeks. The chlorophyll content of mature leaves significantly increased with dry-fog culture only at four weeks.

To evaluate the efficacy of dry-fog culture as a novel aeroponic system, the growth of lettuce was investigated and compared to DFT hydroponic culture. One of the features of dry-fog culture is water conservation. For the systems used in this study, the DFT always needed at least 30 L of liquid fertilizer, while dry-fog culture could fill a chamber with only 1 L of solution because the droplets of dry fog are very fine. In addition, the total amount of liquid fertilizer in a whole dry-fog culture system was less than 10 L. This aspect is an advantage not only in terms of water resource depletion but also because the Styrofoam chamber culturing unit remains light, making it possible to install the system almost anywhere. Chamber units can also be multistaged easily with low costs, meaning this system is suitable for a plant factory or urban farming. Furthermore, because the effort to maintain the chamber units and fertigate plants are also greatly reduced, dry-fog aeroponics is expected to be usable not only for commercial crop production but also for welfare and food education.

Despite using less liquid fertilizer in the rhizosphere for the dry-fog culture, there was no difference in leaf growth in the two different hydroponic techniques, probably due to changes in root morphology and physiology. Root hairs that developed only in the dry-fog culture had a normal increase in the efficiency of nutrient absorption from heterogeneous soil due to a significant increase in the root surface area (Bates and Lynch, 1996; Ma *et al.*, 2001). Root hairs are not needed in DFT hydroponic technique because there is adequate water and liquid nutrients in the root zone. They are not formed in traditional aeroponics because nutrient solutions are sprayed on roots which are always kept wet. In dry-fog culture, because the rhizosphere is almost an air phase, roots have to catch the very fine droplets of nutrient solutions floating in the air in a chamber. Importantly, as an adaptation mechanism, root hairs develop to catch more droplets of fertilizer. In the present study, the significant increase in root growth was observed only at four weeks after planting. Also the respiration rate of the roots was significantly higher in the dry-fog cultures than in the DFT hydroponic system at four weeks (Fig. 5). The respiration rate of roots has a close relationship with water and nutrient absorption in the roots (Hansen, 1980). Indeed, concomitant increases in both root growth and respiration rate in the dry-fog culture were observed, which might result in promotion of water and nutrient absorption and increases of growth and yield. However, no significant increase in leaf growth was observed in dry-fog culture as compared with DFT. Because the growth and respiration activity of roots significantly increased at four weeks and the morphology of new roots developed in the dry-fog culture changed, it may take longer for plant roots to adapt to the dry-fog culture environment as compared with other hydroponic systems. The ascorbic acid content in the leaves also tended to be slightly higher in the dry-fog culture, but there were no significant differences. Leaf ascorbic acid is a secondary metabolite, and its content in leaves increases under environmental stress conditions (Robinson and Bunce, 2000; Reddy *et al.*, 2004, Koyama *et al.*, 2012). Because there is nearly no extra water in the rhizosphere in dry-fog culture, roots might suffer from slightly drought conditions. The increase in secondary metabolites caused by environmental stresses generally comes with a decrease in growth and harvest (Champolivier and Merrien, 1996; Kirda *et al.*, 2004, Koyama *et al.*, 2012). However in the present study, such decreases were not observed. Under drought conditions, the development of root hairs and increase in root surface area are countermeasures to growth depression due to a water shortage. In dry-fog cultures, plants develop root hairs to catch the very fine droplets of nutrient solution, and this development is thought to effect secondary metabolism. Dry-fog culture might be useful to adjust mild drought conditions in rhizosphere by changing the airflow speed at the frequency of atomization, which affect harvest quality (Brix of secondary metabolite content). Furthermore, the atomized dry-fog from the nutrient solution in a chamber fills the rhizosphere space immediately, meaning the rhizosphere environment is changed quickly and drastically. This aspect is expected to lead to new cultivation methods that can increase the content of secondary metabolites by controlling environmental stresses without decreasing growth or harvest.

With regard to the photosynthetic rates of mature leaves grown in the dry-fog culture, significant increases were shown in both ϕ and Max compared with DFT culture. This indicates that the rhizosphere environment of dry-fog culture may affect the photosynthetic ability of lettuce leaves by improving CO_2 assimilation efficiency of mesophyll cells. The content of leaf chlorophyll was significantly increased in the dry-fog culture at four weeks, and this may be considered to be due to improvement in the activity or quantity of photosynthetic light or dark reaction components. The relationship between enhancement of photosynthetic activity and rhizosphere environment in the dry-fog culture remains unclear.

In summary, dry-fog aeroponics enables lettuce plants to grow with less water without sacrificing yield and leaf quality as compared to traditional hydroponics. The lightweight cultivation chamber can decrease the workload for growers and is easy for new growers to maintain. Dry-fog aeroponics posses potential as a novel hydroponic system to promote high-value crop production and yield more than other hydroponic systems by controlling the rhizosphere environment while saving water and nutrients. It is worth noting that the nozzle used in this study can atomize enough dry-fog to fill a much larger chamber (6000×700×450 mm). More study is needed to determine the utility of a dry-fog culture in horticulture. Further research would be helpful to determine rhizosphere environmental conditions (density of dry-fog, frequency of atomizing, flow speed of dry-fog) to maximize plant growth and the amount of secondary metabolites produced. In addition, optimization of the composition and strength of the nutrient solution, especially for dry-fog culture, is needed and could be determined through investigation of the absorption nutrients in the plants.

References

BATES T.R., LYNCH J.P., 1996 - *Stimulation of root hair elongation in* Arabidopsis thaliana *by low phosphorus availability*. - Plant, Cell Environ., 19(5): 529-538.

BIDDINGER E.J., LIU C., JOLY R.J., RAGHOTHAMA K.G., 1998 - *Physiological and molecular responses of aeroponically grown tomato plants to phosphorus deficiency.* - J. Am. Soc. Hortic. Sci., 123(2): 330-333.

CHAMPOLIVIER L., MERRIEN A., 1996 - *Effects of water stress applied at different growth stages to* Brassica napus *L. var*. oleifera *on yield, yield components and seed quality*. - Eur. J. Agron., 5(3-4): 153-160.

CHERIF M., TIRILLY Y., BELANGER R.R., 1997 - *Effect of oxygen concentration on plant growth, lipidperoxidation, and receptivity of tomato roots to Pythium F under hydroponic conditions*. - Eur. J. Plant Pathol., 103(3): 255-264.

FARREN I., MINGO-CASTEL A.M., 2006 - *Potato minituber production using aeroponics: effect of plant density and harvesting intervals*. - Am. J. Potato Res., 83: 47-53.

HANSEN G.K., 1980 - *Diurnal variation of root respiration rates and nitrate uptake as influenced by nitrogen supply*. - Physiol. Plant, 48: 421-427.

HILLEL S., DAVID W.B., 1988 - *Effect of dissolved oxygen concentrations in aero-hydroponics on the formation and growth of adventitious roots*. - J. Am. Soc. Hortic. Sci., 113(2): 218-221.

JING L., YUNWEI Z., 2013 - *An automatic aeroponics growth system based on ultrasonic atomization*. - Appl. Mech. Mater., 288: 161-166.

KIRDA C., CETIN M., DASGAN Y., TOPCU S., KAMAN H., EKICI B., DERICI M.R., OZGUVEN A.I., 2004 - *Yield response of greenhouse grown tomato to partial root drying and conventional deficit irrigation*. - Agric. Water Management, 69(3): 191-201.

KOYAMA R., ITOH H., KIMURA S., MORIOKA A., UNO Y., 2012 - *Augmentation of antioxidant constituents by drought stress to roots in leafy vegetables*. - HortTechnology, 22(1): 121-125.

LIU C.Z., GUO C., WANG Y.C., OUYANG F., 2003 - *Comparison of various bioreactors on growth and artemisinin biosynthesis of* Artemisia annua *L. shoot cultures*. - Process Biochem., 39: 45-49.

MA Z., BIELENBERG D.G., BROWN K.M., LYNCH J.P., 2001 - *Regulation of root hair density by phosphorus availability in* Arabidopsis thaliana. - Plant, Cell Environ., 24: 459-467.

MOHAMMAD A., KHAN A.G., KUEK C., 2000 - *Improved aeroponic culture of inocula of arbuscular mycorrhizal fungi*. - Mycorrhiza, 9(6): 337-339.

REDDY A.R., CHAITANYA K.V., VIVEKANANDAN M., 2004 - *Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants*. - J. Plant Physiol., 161(11): 1189-1202.

RITTER E., ANGULO B., RIGA P., HERRAN C., RELLOSO J., 2001 - *Comparison of hydroponic and aeroponics cultivation systems for the production of potato minituber*. - Potato Res., 44(2): 127-135.

ROBINSON M., BUNCE J.A., 2000 - *Influence of drought-induced water stress on soybean and spinach leaf ascorbate-dehydroascorbate level and redox status*. - Int. J. Plant Sci., 161(2): 271-279.

WEATHERS P.J., ZOBEL R.W., 1992 - *Aeroponics for the culture of organisms, tissues and cells*. - Biotechnol. Adv., 10(1): 93-115.

ZHAO X., LI T.L., SUN Z.P., 2010 - *Effects of prolonged root-zone* CO_2 *treatment on morphological parameter and nutrient uptake of tomato grown in aeroponic system*. - J. Appl. Bot. Food Qual., 83(2): 212-216.