INTRODUCTION

As plants grow, various environmental stressors such as temperature fluctuations, strong light, desiccation, osmotic pressure, and lack of oxygen or minerals around the rhizosphere affect the plants. The time taken for an environmental stressor to affect a plant depends on the type of environmental stress and the extent of influence depends on plant species, plant organ, growth stage, and growth conditions of the affected plant. It has been estimated that the average yield of crops produced by farms is only 21.6% of the record yield of the crops (Boyer, 1982). This difference in crop yield is mainly attributed to abiotic factors such as water deficit, soil nutrient availability and toxicity, and heat stress or chilling/freezing, each of which can combine with other factors to lower crop productivity (Mittler, 2006).

Crops cultivated in a plant factory are less affected by changes in the abiotic environment than crops cultivated on a farm (Gruda, 2009). Therefore, one can always anticipate a stable supply of crops grown in a factory. In addition, these plants are increasingly seen as safe and reliable because they are unaffected by environmental pollution such as by leakage of chemical substances or radioactive materials. Plant factories are classified into those that use sunlight and those that use artificial light. Of these, the type that has a completely controlled light source can cultivate chemical-free vegetables regardless of weather or location because the plant factory is a closed system where all non-biological environmental factors are controlled. Accordingly, the completely controlled type of plant factory may produce the most volume of green vegetables such as spinach or lettuce in the future. However, cost problems have been identified (Takatsuji, 2013).

In the completely controlled type of plant factory, vegetables grow optimally due to the regulation of light and temperature. For example, it has been reported that the content of anthocyanin, a flavonoid in red leaf lettuce, increased on exposure to blue light during growth (Shoji et al., 2010). In addition, previous studies have shown that the quality and value of vegetables are improved when salinity (seawater or table salt) is provided as an abiotic environmental nutrient, as seen in tomatoes (Kitano et al., 2008), onions (Akamatsu, 2007), and broccoli (Lopez-Berenguer et al., 2009). However, there have been few reports documenting the results of cultivation conditions in which salt is added to the growing cultures of green vegetables, which are extremely vulnerable to salt stress, with regard to their impact on the quality of the plants produced in a closed-type plant factory.

In this study, we cultivated lettuce in a hydroponic culture containing either seawater or sodium chloride (NaCl). The quantity of nutrients and pigments in lettuce was measured and compared to attempt to improve the quality and value of lettuce.
MATERIALS AND METHODS

Plant materials and growth conditions

Red leaf lettuce (Lactuca sativa L. cv. Mother-red, Takii Co., Ltd.; Produced in Netherlands) was grown hydroponically for 47 days, and the leaves of the plants were subjected to measurements after harvesting. Five plants were cultivated individually in each hydroponic medium (see below), and three of them were used for measurements of sugar and pigment contents and antioxidative activity. Four individual plants were used for measurement of fresh weight of the shoot, and five individual plants were used for the measurement of photosynthetic activity.

Lettuce seeds were submerged in Otsuka hydroponic culture (see the following section) overnight, and the seeds were germinated on moistened rock wool. Subsequently, the seeds, together with the rock wool, were fixed to styrofoam and planted in the original plant factory shown in Fig. 1. The following three media were used as hydroponic culture media:

Otsuka hydroponic culture—prepared by dissolving 150 g of Otsuka House 1 and 100 g of Otsuka House 2 in 20 L of purified water (Standard hydroponic culture group; Otsuka Chemical Co., Ltd. (currently Otsuka AgriTechno Co., Ltd.; Section (1) in Fig. 1). The electrical conductivity (EC) of the culture was 1.3 dS m⁻¹.

Hydroponic culture supplemented with seawater—prepared by dissolving 4 L of seawater in 16 L of the Otsuka hydroponic culture (seawater hydroponic culture group; Section (2) in Fig. 1; approx. 0.5% salinity). The surface seawater in the Matto Beach Park (N36°30.6', E136°30.3', Ishikawa, Japan) was sampled and used as seawater. EC of the culture was 10.6 dS m⁻¹.

Hydroponic culture containing NaCl (NaCl hydroponic culture group; Section (3) in Fig. 1)—prepared by adding NaCl to the Otsuka hydroponic culture to adjust the final concentration to 0.5%. EC of the culture was 12.6 dS m⁻¹.

The compositions of the three hydroponic culture media are shown in Table 1.

The growth conditions were as follows: room temperature of 20.5 ± 1.5°C, water temperature of 21.0 ± 1.0 °C, relative humidity of 70 ± 10%, the basal part of the plant body, which is above the level of the hydroponic culture medium, was located 15 cm below a fluorescent lamp (photosynthetic photon flux density of 142.5 ± 47.5 μmol m⁻² s⁻¹), and was cultivated under periods of 12 h of light and dark. The pH was kept within a range of pH 7.0 ± 1.0. The EC and pH values were regularly measured by an EC meter (DEC-2, Atago Co., Ltd., Japan) and a pH meter (DPH-2, Atago Co., Ltd., Japan) throughout the cultivation period, respectively, and were maintained stably without replacing the solutions by completely closed hydroponic flow in the factory. In addition, room temperature, water temperature, and humidity were measured every hour using a data logger (TR-72Ui, T&D Co., Ltd., Japan) and were stably managed.

Measurement of sugar, anthocyanin, carotenoid, and chlorophyll concentrations

The sugar content of the third leaf was measured using a Brix meter (PEN-J, ATAGO Co., Ltd., Japan) and expressed in the Brix scale (symbol: °Bx). Briefly, the leaf was homogenized with a mortar and pestle, the homogenate was then filtered through a paper towel, and the filtrate was subjected to measurement of the sugar content using the Brix meter.

The concentration of anthocyanin was measured using young and growing lettuce leaves (the eighth leaves) just after harvesting. The leaf tip (0.2 g) was cut, smashed, and added to 2 mL of 3% HCl/methanol (v/v). Thereafter, the mixture was maintained at 4°C for 18 h, and the absorbance of the extract at 540 nm was measured using a microplate reader (model 680, Bio-Rad Lab. Inc., USA). Cyanidin 3-glucoside chloride (Funakoshi Co., Ltd., Japan) was used for preparing the calibration curve.

The concentrations of carotenoid and chlorophyll were measured using the seventh leaves. A leaf (2 g) was placed into a mortar with 3 mL of 100% acetone, and homogenized with a pestle. The homogenate was filtered through

<table>
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<tr>
<th>Constituent/ion</th>
<th>Standard</th>
<th>Seawater</th>
<th>NaCl</th>
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<tr>
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<tr>
<td>chloride</td>
<td>0</td>
<td>3806</td>
<td>5000</td>
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EC (dS m⁻¹) 1.3 10.6 12.6

The values were calculated by using the concentrations of the major constituents/ions in seawater according to Taiz and Zeiger (2004) and the concentrations of the minor elements in seawater according to Nozaki (1997).
Miracloth (Merck Millipore Co., Japan), and the pigment in residual tissue was re-extracted with 2 mL of 80% acetone. The filtrate was centrifuged at 15,000 rpm at 4°C for 5 min, and the absorbances of the supernatant at 475 nm, 645 nm, and 663 nm (A475, A645, and A663, respectively) were measured. Concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), and total carotenoids (Cc) were calculated using the following formulae (Lichtenthaler and Wellburn, 1983):

\[
\text{Chl a} = 12.21 \times A_{663} - 2.81 \times A_{645} \\
\text{Chl b} = 20.13 \times A_{663} - 5.03 \times A_{645} \\
C_{c} = (1000 \times A_{663} - 3.27 \times \text{Chl a}) - 104 \times \text{Chl b})/227
\]

Measurement of photosynthetic activity

Fluorescence of Chl a was measured as an index of photosynthetic activity. Leaves of the plants were used to measure the fluorescence of Chl a before harvesting. For Chl a fluorometry, the leaves were covered with a dark leaf clip of a plant efficiency analyzer (Pocket PEA; HansaTech Instruments, Ltd.) for at least 20 min. Thereafter, the leaf was illuminated with a red light pulse (2,500 mol m⁻² s⁻¹, peak wavelength: 627 nm), and the change in fluorescence at 1 sec after illumination was measured. The Fv/Fm value, which indicates the maximum quantum efficiency of photosystem II, was defined as a parameter of photosynthetic activity.

Measurement of antioxidative activity

Lettuce leaves were used to measure the antioxidative activity. One leaf was homogenized in a mortar and pestle, filtered through a paper towel, and 20 μL of the filtrate was used to measure the antioxidative activity using a “Radical Catch” measurement kit (Hitachi Aloka Medical, Ltd.). A luminometer (AccuFlex Lumi400; Hitachi Aloka Medical, Ltd. or GENE LIGHT GL-210; Microtec Co., Ltd.) was used to measure the amount of chemiluminescence, and a multiplication value of 90 sec of the chemiluminescence was measured.

Statistical analyses

The mean values of fresh weights of shoot, contents of sugar and pigments, and photosynthetic and antioxidative activities were compared by using the Tukey’s honest significant difference (HSD) test to identify significant differences at the 5% and 1% levels.

RESULTS

Size and sugar content of lettuce

The appearance of lettuce plants grown in hydroponic culture for 47 days in our plant factory is shown in Fig. 2. No obvious differences were observed in the sizes of lettuce plants cultivated in seawater hydroponic culture and those cultivated in hydroponic culture (Fig. 2a and b). On the other hand, the lettuce plants cultivated in the NaCl hydroponic culture were smaller than those of the above two groups (Fig. 2c). In addition, the leaves of lettuce plants cultivated in the seawater-based hydroponic culture and NaCl hydroponic culture showed more intense red color compared with the leaves cultivated in standard hydroponic culture. No such obvious difference between groups was observed in the degree of green coloration.

The fresh weight of shoots and sugar content of leaves grown in each cultivation group were measured. The results are shown in Fig. 3. Although no significant differences in the fresh weight of lettuce were observed between the standard hydroponic culture and seawater hydroponic culture, the fresh weight of lettuce cultivated in the NaCl hydroponic culture was remarkably low. Significant differences were observed between the standard hydroponic culture and the NaCl hydroponic culture (P < 0.01), as well as between the seawater hydroponic culture and the NaCl hydroponic culture (P < 0.05; Fig. 3a). The sugar content of the lettuce cultivated in the standard hydroponic culture was approximately 2.5%, whereas that of the lettuce cultivated in seawater hydroponic culture and NaCl hydroponic culture was approximately 4% (Fig. 3b). Significant differences (P < 0.05) in the value of sugar content were observed among all of the hydroponic culture groups.

Pigment concentrations

The concentration of anthocyanin in the lettuce leaf cultivated in each hydroponic culture condition is shown in Fig. 4. The graph pattern that results from the measurements of anthocyanin content was similar to the graph pattern of the sugar content (Fig. 3b). The concentration was approximately 200 μg g⁻¹ in standard culture and approximately 400 μg g⁻¹ in seawater and NaCl hydroponic cultures. Significant differences (P < 0.05) were observed between the standard hydroponic culture and NaCl hydroponic culture.
The concentrations of chlorophylls a and b are shown in Fig. 5a, and the concentration of total carotenoid is shown in Fig. 5b. Lettuce cultivated in seawater and NaCl hydroponic cultures showed higher concentrations of both chlorophyll a and b, compared to concentration of standard hydroponic culture. For chlorophyll a, a significant difference was observed between seawater and standard hydroponic cultures ($P < 0.05$). For chlorophyll b, significant differences were observed between seawater and standard hydroponic cultures ($P < 0.01$), and between seawater and NaCl hydroponic cultures ($P < 0.05$). Carotenoid concentrations were, in order from highest to lowest, as follows: lettuce cultivated in seawater hydroponic culture, standard hydroponic culture, and NaCl hydroponic culture. A significant difference ($P < 0.05$) was observed between seawater and NaCl hydroponic cultures.

Photosynthetic activity and antioxidative activity

Since differences in the contents of chlorophyll a and b were seen among cultivation groups, the quantum yield $Fv/Fm$ of photosystem II, an index of photosynthetic activity, was measured. The result is shown in Fig. 6. The values in the cultivated lettuces were, in order from highest to lowest, as follows: NaCl, seawater, and standard hydroponic culture. A significant difference ($P < 0.05$) was observed between NaCl and standard hydroponic cultures.

The antioxidative activities of lettuce extracts from the seawater and NaCl hydroponic cultures were significantly higher than that of standard hydroponic culture (Fig. 7). Significant differences were observed between seawater and standard hydroponic cultures, and between NaCl and standard hydroponic cultures ($P < 0.01$). Since differences in pigment content and photosynthetic/antioxidative activities were observed, protein concentration and composition in lettuce extracts were examined. The protein concentrations were, in order from highest to lowest, as follows: NaCl, seawater, and standard hydroponic culture (data not presented).
EFFECT OF SEA WATER ON LETTUCE GROWTH

The size of red leaf lettuce plants was decreased and their fresh weights were remarkably decreased by the addition of NaCl to the hydroponic culture (Figs. 2 and 3a). The NaCl concentration of the NaCl hydroponic culture in this study was 0.5% (i.e., a molar concentration of approximately 90 mM). It has been reported that in the growth of peas, which are susceptible to salinity like lettuce, the fresh weight decreased to 46.3% of the expected weight in standard medium, because of competition between the inhibitory effect on growth by NaCl and the stimulatory effect on growth by nutrients in the seawater, the size of the shoot and fresh weight were virtually identical to those of plants cultivated in additive-free medium. Another explanation for this result is that the salinity in seawater hydroponic medium was moderate for leaf lettuce to grow, resulting in fewer reductions in size and fresh weight, as seen in the case of ro- maine lettuce (Kim et al., 2008). There were no significant differences in heights and appearances of the roots grown in standard, seawater, and NaCl hydroponic media (data not shown).

The sugar content of lettuce leaves grown in the media containing NaCl or seawater was approximately twice as high as that of lettuce grown in the additive-free medium (Fig. 3b). It has been reported that sugar contents increase through cultivation in the presence of salinity in tomatoes (Kawai et al., 2002; Saito et al., 2006; Kitano et al., 2008) and in Japanese radish root (Shinohara and Kagiwada, 2008). We assume that lettuce plants are subjected to osmotic stress by the addition of salt to hydroponic culture, and as a result, sugars are produced and accumulate in the cells of these leaves as a compatible solute. Compatible solutes such as sugars and some amino acids function as osmolytes and are believed to facilitate osmotic adjustment by acting as osmoprotectants (Hasegawa et al., 2000).

The synthesis of anthocyanin is promoted when plants are exposed to variables such as salinity and sugars (Chalker-Scot, 1999). Therefore, it is assumed that the anthocyanin content is markedly increased in lettuce leaves that are exposed to salinity stress or sugars. Anthocyanin was accumulated in seedlings because of exposure of plants to salinity stress (Eryilmaz, 2006). Increased reactive-oxygen-scavenging activity was observed in the extracts of lettuce grown in cultures with seawater or NaCl added (Fig. 7). This may be due to the contribution of anthocyanin accumulated in the leaf.

An approximately 40% increase in the concentration of chlorophylls a and b and β-carotene was observed in cotton plants provided with seawater at a concentration of 26% (Brugnoli and Bjorkman, 1992). On the other hand, the total chlorophyll content per fresh weight decreased to half in spinach to which 200 mM of NaCl was continuously provided (Downton et al., 1985). Spinach is vulnerable to salinity and cotton shows intermediate tolerance to salinity shown). However, separation of proteins by SDS-PAGE and subsequent CBB staining revealed no obvious differences in the amounts of the major proteins or the band pattern of the proteins (data not shown).

DISCUSSION

The size of red leaf lettuce plants was decreased and their fresh weights were remarkably decreased by the addition of NaCl to the hydroponic culture (Figs. 2 and 3a). The NaCl concentration of the NaCl hydroponic culture in this study was 0.5% (i.e., a molar concentration of approximately 90 mM). It has been reported that in the growth of peas, which are susceptible to salinity like lettuce, the fresh weight decreased to 46.3% of the expected weight in Challis cultivars and 78.7% of the expected weight in Granada cultivars by the addition of 70 mM NaCl (Hernandez et al., 1995). In addition, the fresh weights of romaine lettuce, which is more tolerant to salt stress than leaf lettuce, were dramatically decreased when treated with NaCl at 100 mM concentration (Kim et al., 2008; Mahmoudi et al., 2010). Therefore, it is suggested that the inhibitory effect on plant growth was observed in conditions of 100 mM NaCl or less, for plants susceptible for salinity stress. Considering that plant cell division is not significantly affected by salinity (Ogawa et al., 2006), and that reduction in dry weight of romaine lettuce treated with 100 mM NaCl was far less than reduction in fresh weight of the plant (Mahmoudi et al., 2010), the growth of the cells in lettuce leaves under saline conditions seems to be inhibited. On the other hand, there were no significant differences between the sizes (Fig. 2) and the fresh weights (Fig. 3a) of lettuce plants cultivated in seawater and standard media. It is generally known that when seawater is provided to plants during cultivation, minerals contained in the seawater may stimulate growth (Kawai et al., 2002; Akamatsu, 2007; Kitano et al., 2008; Islam et al., 2010). In lettuce plants cultivated in the seawater hydroponic medium, because of competition between the inhibitory effect on growth by NaCl and the stimulatory effect on growth by nutrients in the seawater, the size of the shoot and fresh weight were virtually identical to those of plants cultivated in additive-free medium. Another explanation for this result is that the salinity in seawater hydroponic medium was moderate for leaf lettuce to grow, resulting in fewer reductions in size and fresh weight, as seen in the case of ro- maine lettuce (Kim et al., 2008). There were no significant differences in heights and appearances of the roots grown in standard, seawater, and NaCl hydroponic media (data not shown).

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(Brugnoli and Bjorkman, 1992; Shannon and Grieve, 1999). As a result of cultivating lettuce, which is vulnerable to salinity, in hydroponic cultures containing 20% (100 mM NaCl equivalent) seawater in this study, marked increases in the chlorophyll and carotenoid contents were observed. In addition, a significant increase in chlorophyll concentration was observed in lettuce cultivated by the addition of NaCl (Fig. 5). The decrease in size and fresh weight of lettuce plants grown in NaCl hydroponic culture suggests that NaCl inhibits cell growth, but does not inhibit the synthesis and accumulation of photosynthetic pigments. On the other hand, the size and fresh weight of lettuce grown in seawater hydroponic culture were almost the same as those in the control culture, and the content of chlorophyll and carotenoids were increased. Thus, it suggests that some nutrient contained in seawater may enhance the synthesis and accumulation of chlorophylls and carotenoids. The quantum yield of photosystem II was the highest in the NaCl hydroponic culture followed by that in seawater; a significant difference was observed between NaCl and control cultures (Fig. 6). These results indicate that the degree of increase in photosynthetic pigments does not directly increase photosynthetic activity. Thus, not only an increase in harvested light energy reflects reaction efficiency of photosystem II but also other whole functions of the plant is coordinated, such as the negative control of gene expression for the constitutive proteins of photosystem II by salinity stress, synthesis of osmolytes and ion channels induced by osmotic and ion stresses, and adaptation to oxidative stress induced by salinity stress (Zhu, 2001; Chaves et al., 2009).

The hydroponic medium supplemented with seawater contained higher amounts of potassium, calcium, magnesium, and borate ions than standard medium (Table 1). Among these ions, the potassium ion is one of the most essential elements for plants, and calcium ion has a role in providing salt tolerance to plant cells (Hu and Schmidhalter, 2005; Mahajan and Tuteja, 2005). There are few reports dealing with the effects of trace elements on the plants when the plants were subjected to NaCl salinity (Stamatakis et al., 2003; Hu and Schmidhalter, 2005). Likewise, silica and the other micronutrients may suppress the inhibitory effect of NaCl salinity on growth of lettuce plants.

In this study, the hydroponic cultivation of lettuce, which is a leafy green crop, particularly using a medium containing seawater, enables the production of high quality lettuce, superior in both its nutritional and functional aspects. Therefore, the use of seawater for hydroponic culture may be applicable to cultivation of green vegetables in general plant factories.

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REFERENCES


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