INTRODUCTION

Zinc (Zn) is required by nearly all plants and animals as it is a vital component of many proteins and enzymes (Hacisalihoglu and Kochian, 2003). Zn plays an important role in homeostasis, apoptosis, aging, oxidative stress, and immune functioning in animals, including humans (Chasapis et al., 2012), and Zn deficiency is one of the major health problems for humans globally, including in Japan (Kogirima et al., 2007). Zn is mainly taken up by plant roots as the Zn$^{2+}$ ion (von Wirén et al., 1996) and the total Zn concentration and free Zn$^{2+}$ activity in the soil can vary greatly depending on a number of soil factors (e.g., pH, organic matter content, soil moisture). However, insoluble Zn comprises over 90% of the soil Zn and is unavailable for plant uptake, and in calcareous soil with a high pH, the free Zn$^{2+}$ activity can be as low as $10^{-11}$–$10^{-7}$ M, which can be too low to support optimal crop growth (Hacisalihoglu and Kochian, 2003).

One approach for addressing Zn deficiency is the biofortification of hydroponically grown vegetables with Zn, which has recently been shown to be more practical and effective than dietary diversification, supplementation, or food fortification (White and Broadley, 2011; Sago et al., 2018). In hydroponic culture, plants are grown using nutrient solution (water and fertilizer) with or without an artificial medium. Such a system avoids the need for the costly and time-consuming task of soil sterilization to prevent soil-borne diseases and enables precise fertilizer management (Wahome et al., 2011; Lakskireddy et al., 2012), bringing numerous benefits to both producers and consumers. Hydroponics is also effective in modifying the nutrient composition of vegetables—for example, it can be used to reduce the nitrate content (Wang et al., 2007; Stefanelli et al., 2005)—for example, their edible stems are widely consumed as a vegetable in Mexico, Latin America, South Africa, and Mediterranean countries (Stintzing and Carle, 2005; Cruz-Hernández and Paredes-López, 2010; El-Mostafa et al., 2014). In some countries, these plants are also used as a remedy for a variety of health problems, including edema and indigestion (El-Mostafa et al., 2014). Edible cacti are produced on a small scale in Japan, mainly in Kasugai City, Aichi Prefecture, and it has previously been shown that the edible cactus \textit{Nopalea cochenillifera} (L.) Salm-Dyck can be grown in a simple hydroponic culture system using commercially available materials (Horibe and Yamada, 2016; Horibe, 2017) and can tolerate various concentrations of heavy metals (Horibe et al., 2019). However, to our knowledge, few studies have investigated the relationship between the Zn concentration in nutrient solution and growth of edible cacti under hydroponic conditions. Therefore, in this study, we investigated how supplementing the nutrient solution with different concentrations of Zn affected the growth and Zn accumulation of hydroponically grown \textit{N. cochenillifera}.
MATERIALS AND METHODS

Plant materials
Cladodes of *N. cochenillifera* (16 × 6.5 × 1 cm) were harvested at a commercial cactus farm (Goto saboten) in Aichi Prefecture, Japan, in June 2018. Within 1 h of collection, the cladodes were transported in a dry condition to our laboratory at Chubu University (Kasugai, Japan) and were trimmed to a length of 15 cm.

Heavy metal treatments

The harvested cladodes were grown hydroponically in a greenhouse at Chubu University using the deep flow technique (Horibe, 2017). Briefly, each cladode was attached to a 4.5-L plastic vessel using an L-shaped plastic bar (L-type angle) and clips and the vessel was then filled with OAT House solution A (electrical conductivity=2 dS/m, pH=6.5; OAT Agrio Co., Ltd., Japan), which was prepared by dissolving 150 g of OAT House 1 and 100 g of OAT House 2 in 100 L of water. OAT House solution A used in this experiment contains 0.09 ppm (w/v) Zn. Zinc nitrate [Zn(NO₃)₂]₃ was then added to the nutrient solution in each vessel to give concentrations of 0, 50, and 200 ppm (w/v) Zn. Deionized water was added to the vessels once per week to replace any evaporated water.

The experiment was conducted for 10 weeks from June 25 to September 3, 2018. During the experimental period, the maximum and minimum temperatures were measured daily by a data logger (TR-71wb; T&D Co., Ltd., Japan). The average highest and lowest temperatures during experiment were 42.7˚C and 25.3˚C in June, 38.7˚C and 26.9˚C in July, 39.7˚C and 27.0˚C in August, 34.2˚C and 23.9˚C in September. The number of daughter cladodes and the length of the first daughter cladode on each mother plant were measured each week. At 10 weeks after the start of treatment, each plant was dissected into daughter cladodes, above-ground parts of the mother cladode, underwater parts of the mother cladode, and roots, and the fresh weight (FW) of each plant part was measured. We dissected mother cladodes into above-ground parts and underwater parts. Because we wanted to evaluate the effect of direct contact with nutrient solution containing different concentration of Zn on their growth by comparing these two parts. The harvested roots were then dried at 65˚C for 72 h, while the harvested daughter and mother cladodes were dried at 65˚C for 144 h.

Sample measurement

The dried plant parts were dissolved in nitric acid (HNO₃) and heated until the organic matter was completely dissolved. The dissolved sample solution was then filtered through filter paper and the Zn²⁺ concentration was measured using atomic absorption spectrometry (AAAnalyst400; PerkinElmer Japan Co., Ltd., Japan) following standard procedures.

Experimental design and statistical analysis

Six cladodes were used for each treatment and four cladodes per treatment were used for sample measurement. Differences between treatments were analyzed using analysis of variance and differences between the means were determined using Tukey’s test with a significance level of \( P=0.05 \).

RESULTS AND DISCUSSION

The availability of metal ions in the soil is strongly affected by a number of physical and chemical soil properties because the transfer of metals between the solid and solution phases is influenced by competition from other cations for surface exchange sites and the presence of binding surfaces, such as organic matter (Martin and Kaplan, 1998; Rieuwerts et al., 2006; Manousakis and Kalogerakis, 2009). Hydroponics overcomes this barrier and allows precise fertilizer management, leading to improved crop quality (Sakamoto et al., 1999).

In the present study, daughter cladodes developed from mother cladodes in all treatments (Figs. 1 and 2). The...
number of daughter cladodes increased rapidly during the first 3 weeks of treatment and remained almost stable after 6 weeks, with no significant difference among treatments (Fig. 2B). However, while the length of the first daughter cladodes increased until the end of the experiment with all three treatments, they were significantly shorter with the 200 ppm treatment (Fig. 2A). Furthermore, the FWs of the daughter cladodes and underwater parts of the mother cladodes were significantly lower with the 200 ppm treatment than in the control plants (Table 1). Together, these findings suggest that a high concentration of Zn in the nutrient solution negatively affected the growth of the daughter and mother cladodes, as well as the harvestable biomass. Interestingly, the FW of the roots was significantly higher with the 50 ppm treatment than with the 0 or 200 ppm treatments (Table 1), indicating that moderate amounts of Zn may promote root elongation in N. cochenillifera, although the precise mechanism by which this could occur is currently unknown.

Supplementation of the nutrient solution with Zn led to a drastic increase in the concentration and amount of accumulated Zn in the various plant parts (Table 1). The concentrations of Zn in the underwater parts of the mother cladodes and the roots were significantly higher with the 200 ppm treatment than with the 50 ppm treatment despite the amount of accumulated Zn in these plant parts not differing between treatments (Table 1), which can be explained by the inhibited growth and lower biomass with the 200 ppm treatment. When we compared Zn concentration between the above-ground and the underwater parts of the mother cladodes, it became higher in the underwater parts under the 200 ppm treatment (Table 1). Since Zn in nutrient solution enter from root and/or underwater parts of mother cladodes, we presumed that Zn concentration of underwater parts became higher compared with above-ground parts. However, it became higher in the above-ground parts under control treatment, and there was no significant difference between them under 50 ppm treatment (Table 1). Thus, more research is needed to understand Zn transportation and compartmentation mechanism in cladodes.

Most crop plants require leaf Zn concentrations greater than 15–30 μg g⁻¹ dry weight (DW) for maximal yield, and their growth is inhibited at leaf Zn concentrations greater than 100–700 μg g⁻¹ DW (White and Broadley, 2011). The daughter cladodes, which represent the harvestable part in commercial cultivation, contained 1.212 μg Zn g⁻¹ DW with the 50 ppm treatment and 1.542 μg Zn g⁻¹ DW with the 200 ppm treatment (Table 1), which are higher concentrations than have previously been reported in Zn biofortification studies on leaf lettuce (about 7.0 mg Zn 100 g⁻¹ FW) (Sago et al., 2018) and other leafy vegetables (about 265 μg Zn g⁻¹ DW) (Barrameda-Medina et al., 2017). We also calculated that >8.0 mg/100 g FW of Zn accumulated in the daughter cladodes with both the 50 ppm and 200 ppm treatments (Table 1), which means eating 100 g of these cacti can satisfy the recommended daily amount of Zn (8.0 mg) but within the tolerable upper intake level (40 mg/day) (White and Broadley, 2005). Thus, our results show that the Zn concentration of N. cochenillifera can be increased by supplementation of the hydroponic nutrient solution with Zn and that N. cochenilli-fera can accumulate higher amounts of Zn in the above-ground body parts than other Zn-biofortified vegetables. Furthermore, with both the 50 ppm and 200 ppm treatments, the Zn concentration in the roots was much greater than 7.0 mg Zn g⁻¹ DW with the 50 ppm treatment and 1,542 μg Zn g⁻¹ DW with the 200 ppm treatment (Table 1), which means eating 100 g of these cacti can satisfy the recommended daily amount of Zn (8.0 mg) but within the tolerable upper intake level (40 mg/day) (White and Broadley, 2005).}

### Table 1: Effect of Zinc (Zn) treatment on cladode growth and Zn accumulation in plant parts.

| Plant part          | Treatment | Fresh weight (g) | Zinc conc. (μg/g DW) | Zinc amount (μg) | Zinc conc. (mg/100 g FW) |
|---------------------|-----------|------------------|----------------------|-----------------|--------------------------|
|                     |           |                  |                      |                 |                          |
| Daughter cladode    | control   | 52.3 ± 2.4        | a                    | 93.5 ± 3.8      | a                        | 0.6 ± 0.2                |
|                     | 50 ppm    | 53.9 ± 1.8        | a                    | 1212 ± 118.5    | b                        | 4375 ± 419.1             | 8.1 ± 0.7                |
|                     | 200 ppm   | 24.7 ± 2.4        | b                    | 1542 ± 62.6     | b                        | 2376 ± 442.1             | 10.4 ± 0.4               |
| Mother cladode (above-ground) | control | 30.7 ± 0.6        | ab                   | 108.9 ± 11.2    | a                        | 217.7 ± 24.6             | a                        |
|                     | 50 ppm    | 33.1 ± 1.5        | a                    | 641.5 ± 63.6    | b                        | 1454.7 ± 243.2           | a                        |
|                     | 200 ppm   | 24.8 ± 2.2        | b                    | 744.9 ± 22.7    | b                        | 1161.9 ± 180.2           | a                        |
| Mother cladode (underwater) | control | 21.7 ± 0.9        | a                    | 71.6 ± 7.8      | a                        | 100.8 ± 4.8              | a                        |
|                     | 50 ppm    | 22.1 ± 0.8        | a                    | 656.4 ± 31.3    | b                        | 956.2 ± 22.9             | b                        |
|                     | 200 ppm   | 14.9 ± 1.1        | b                    | 1048.8 ± 28.1   | c                        | 1063.3 ± 59.6            | b                        |
| Roots               | control   | 1.07 ± 0.09       | a                    | 25.4 ± 62.3     | a                        | 15.5 ± 4.2               | a                        |
|                     | 50 ppm    | 2.08 ± 0.21       | b                    | 4909.1 ± 434.0  | b                        | 694.5 ± 53.9             | a                        |
|                     | 200 ppm   | 0.67 ± 0.12       | a                    | 14566.1 ± 3022.6| c                        | 562.5 ± 69.3             | a                        |

Different letters indicate significant differences among treatments in same plant part (least significant difference test, P<0.05).

Average ± SE (n=6 for fresh weight, n=4 for Zinc conc. and Zinc amount).
Phytoremediation, which is the use of plants to remove pollutants from the environment, has been proposed as a cost-effective, environmentally friendly alternative for the restoration of heavy metal-contaminated soils (Palmer et al., 2001; Butcher, 2009; Ouederje and Babalola, 2017) and several studies have suggested that desert plants represent suitable candidates for phytoremediation (Figuerola et al., 2007; Buendía-González et al., 2010; Adki et al., 2013). Adki et al. (2013) previously reported that *N. cochenillifera* shows a high adaptability to different environmental conditions in arid and semi-arid climates and is a hyperaccumulator of chromium (Cr) and more recently, Horibe et al. (2019) found that this plant can tolerate various concentrations of cadmium (Cd), lead (Pb), and Zn under hydroponic conditions. In the present study, we further demonstrated that *N. cochenillifera* is also a hyperaccumulator of Zn. Therefore, since this cactus produces high biomass and can easily be propagated vegetatively (Zutta et al., 2011), we believe that it is also a good candidate for the phytoremediation of heavy metals in arid regions in addition to its current role as a source of healthy food and fodder.

In this study, we demonstrated that the supplementation of nutrient solutions with Zn affects the growth and Zn concentration of *N. cochenillifera* and that this cactus has a high capacity for accumulating Zn. However, Zn has been shown to interact with other elements (Kabir et al., 2014)—for example, phosphorus (P) interferes with Zn acquisition, so that high levels of P decrease the availability of Zn and P fertilizer application is associated with the opposite. Zn deficiency (Messawie et al., 2012, Kabir et al., 2014). Thus, the uptake of Zn by *N. cochenillifera* will be affected by its interaction with other elements as well as other environmental conditions, such as temperature, pH, and photoperiod. Therefore, further research is required to better understand the relationship between Zn uptake and cultivation conditions in edible cacti.

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