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Response of Common Ice Plant (*Mesembryanthemum crystallinum* L.) to Sodium Chloride Concentration in Hydroponic Nutrient Solution

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Abstract. **Common ice plant (*Mesembryanthemum crystallinum* L.) is a novel edible succulent plant with savory flavor. It has epidermal bladder cells (EBCs) that store water and sodium chloride (NaCl) located on the epidermis of the leaves and stems. Ice plant is an obligatory halophyte that requires NaCl for optimum growth. The objective of this study was to determine the impact of NaCl on growth of ice plant for hydroponic production as an edible leafy green and to quantify the ability of ice plant to take up NaCl from the environment. Four-week-old seedlings of ice plant were transplanted into hydroponic systems, established for 1 week, and given five NaCl treatments [0 M (control), 0.05 M, 0.10 M, 0.20 M, 0.40 M NaCl]. Sequential destructive harvests to determine plant growth occurred at day 7, 14, and 21 after NaCl treatment. The 0.05 M NaCl had the greatest stimulating effect on biomass, increasing total fresh weight (FW) by 173% and shoot FW by 193% compared with the control plants. The 0.10 M NaCl also had stimulating effect as compared with 0 M, but plants were not as large as those receiving 0.05 M NaCl. The 0.20 M NaCl had little effect on plant growth compared with the control. The 0.40 M NaCl had a strong stunting effect on plant growth. All plants treated with NaCl had less root weight than the control, and higher NaCl concentration resulted in greater reduction in root weight. However, for the 0.05 and 0.10 M treatment, the gain in shoot weight exceeded the loss in root weight. Plants gained or lost water in a faster rate than dry mass, which resulted in larger differences among treatments in FW than in dry weight (DW). Plants treated with higher NaCl concentrations developed fewer, smaller, and thicker leaves but contained more EBCs per unit leaf surface area. There was high Na and Cl accumulation in leaf tissues of all salt-treated plants (e.g., 180,507 mg·kg^{-1} Na and 125,084 mg·kg^{-1} Cl in the 0.05 M treatment vs. 13,558 mg·kg^{-1} Na and 12,991 mg·kg^{-1} Cl in the 0 M treatment). This indicated potential for bioremediation of saline soil or hydroponic water. Concentrations of macronutrients such as nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg), and sulfur (S) were reduced when plants received increasing NaCl treatments. In general, this study showed that growth of ice plant benefited from 0.05 and 0.10 M NaCl additions to the hydroponic nutrient solution. Ice plant deserves further work on its ability to reduce Na and Cl from accumulating in recirculating hydroponic nutrient solution.**

Common ice plant (*Mesembryanthemum crystallinum* L.) is an edible succulent plant emerging as a new ingredient for salad. Ice plant has a high nutritional value for humans due to its abundant antioxidant compounds, such as phenolic compounds (Kang et al., 2016). Ice plant is used as food and in medical treatment and therapeutic cosmetics (Loconsole et al., 2019). As hydroponics and controlled environment technologies become more widely used for the production of fresh and high-quality vegetables, greenhouse growers are looking to expand the crops they produce, and some have added ice plant into their production mix.

Ice plant is known for its ability to take up sodium chloride (NaCl) and stores water and NaCl in epidermal bladder cells (EBCs) (Agarie et al., 2007). This characteristic not only brings appealing salty and succulent (i.e., juicy) flavor but implies potential of this plant to deal with salinization of soil and water. In the halophyte database produced by Menzel et al. (2003), ice plant is categorized as an obligatory halophyte, which requires saline environments for optimal growth. That explains the fact that ice plants are typically found on coastal sand dunes, saline flats, and inland saline areas (Loconsole et al., 2019). Although there is debate about the NaCl threshold defining halophytes, Flowers and Colmer (2008) define halophytes as plants that survive to reproduce in an environment where salt concentration is around 0.2 M NaCl or more. Halophytes have developed salt-adapting mechanisms, such as ion homeostasis through ion extrusion and compartmentalization, osmotic adjustments, and antioxidant production (Joshi et al., 2015). Ice plant as a halophyte transports NaCl into its special cells (i.e., EBCs) on the leaf surface, resulting in low NaCl concentrations in photosynthetically active leaf tissues (Agarie et al., 2007). Further, ice plant switches from C3 metabolism to Crassulacean acid metabolism when exposed to high salinity as a strategy to prevent water loss (Winter et al., 1982). Abscisic acid plays a critical role in this NaCl stress response (Chu et al., 1990). For ice plant, increased NaCl concentration is reported to increase the accumulation of pinitol and ononitol, compounds that promote human health, with maximum accumulation at 0.40 M NaCl concentration (Agarie et al., 2009).

Previous research has focused on mechanisms of ice plant's salt tolerance through metabolic mechanisms but lacks applied information that correlates salt concentration with hydroponic productivity (biomass and plant morphology). From an agronomic perspective, NaCl is added to the fertilizer to promote growth and savory flavor in ice plant production. However, limited information is available to inform the effect of NaCl concentration on plant growth and performance. Research conducted by Agarie et al. (2007) exposing ice plants to 0, 0.1, 0.2, 0.4, and 0.8 M NaCl showed that highest dry weight (DW) accumulation of ice plant occurred between 0.1 and 0.2 M NaCl treatment. However, plants were not exposed to saline treatments until they were 45 d old, and the focus of the study was on comparing the reproductive potential of wild-type with EBC-less mutant plants. Ice plants used as edible leafy greens are typically harvested within 6–8 weeks from sowing seeds. More information is needed on the biomass response of the crop to earlier NaCl treatments during hydroponic production as a leafy green crop.

In standard hydroponic crops [tomato (*Solanum lycopersicum* L.), cucumber (*Cucumis sativus* L.), lettuce (*Lactuca sativa* L.), and cut flowers], NaCl is not used in large quantities by the plant, so it can accumulate over time and hinder the absorption of essential elements such as N, K, Ca, and Mg (Neocleous and Savvas, 2017). The use of tap water (which typically has some NaCl) rather than deionized/reverse-osmosis water makes this phenomenon worse because the hydroponic nutrient solution is captured and reused, with a resulting increase in NaCl concentration over time and eventually becoming limiting for plant growth (Voogt and Van Os, 2010). Replacement of the hydroponic

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solution is required to solve this problem, but fertilizer wastewater results in excessive nutrient pollution, especially nitrate and phosphorus pollution (Kumar and Cho, 2014). Incorporating ice plants into other hydroponic growing systems, such as lettuce systems, may mitigate or eliminate the accumulation of NaCl, resulting in less frequent replacement of the hydroponic solution. For the treatment of hydroponic wastewater, constructed wetlands have also been used to remove polluting elements such as N and P (Gagnon et al., 2010). Incorporating ice plant into constructed wetlands may effectively remove Na and Cl as well. However, research is lacking on the quantity of Na, Cl, and other mineral elements that are accumulated by ice plant in response to NaCl concentration in the hydroponic solution over time. Therefore, the objectives of this project were to determine 1) the response of ice plant (yield and morphology) to NaCl concentration in the hydroponic solution to inform production practices and 2) the extent to which ice plant can accumulate NaCl for future applications mitigating NaCl accumulation in recirculating hydroponic systems.

Materials and Methods

Plant material. Seeds of ice plant (Baker Creek Heirloom Seeds, Mansfield, MO) were started in 2.5-cm rockwool cubes that were pre-soaked with 21N–2.2P–16.5K Jack's All Purpose Fertilizer (JR Peters, Allentown, PA) at a concentration of 150 mg·L^{-1} N and placed in trays (Fig. 1). For crop cycles when seeds were germinated in February and March, the plug trays were placed on a heat mat maintaining a 20 °C rockwool temperature. All seed trays were fertilized daily with the aforementioned fertilizer solution and raised in a controlled greenhouse environment with 19.8 ± 0.5 °C (mean ± SD) day temperature and 19.0 ± 0.6 °C (mean ± SD) night temperature. A photoperiod of 20 h·d^{-1} and supplemental lighting were supplied by high-pressure sodium fixtures set to provide 55 μmol·m^{-2}·s^{-1} at the plant canopy. Germination generally occurred 2–3 d after sowing, and seedlings were thinned to one per rockwool cube 3 weeks after sowing. The entire germination stage took 4 weeks. The seedlings were then selected for uniformity (Fig. 2) and transplanted to the experimental setting.

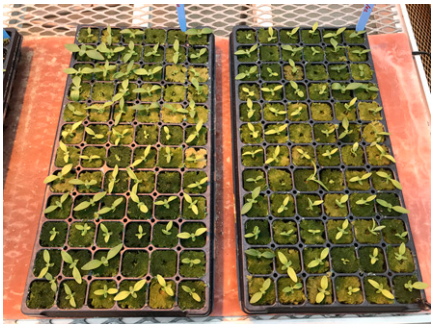

Fig. 1. Seedlings of ice plant grown in rockwool before hydroponic experiments.

Experimental setting. Mini-pond hydroponic systems were created using 4-L containers filled with nutrient solution (Fig. 3). A 2.5-cm hole was drilled on the cover of each container to fit the rockwool cube. Each single seedling was transplanted into one container with the bottom of the rockwool cube and plant roots touching the nutrient solution. Air pumps supplied air to the nutrient solution through tubing connected to air stones placed into the container to maintain saturated dissolved oxygen. All plants were grown in controlled greenhouse environment with 22.5 ± 0.7 °C (mean ± SD) day temperature, 21.2 ± 0.8 °C (mean ± SD) night temperature, and a 14-h photoperiod. High-pressure sodium lights provided 88.9 ± 9.8 μmol·m^{-2}·s^{-1} (mean ± SD) as measured at plant canopy. Supplemental light was on from 0600 to 2000 HR daily, which provided a supplemental daily light integral of 4.48 mol·m^{-2}·d^{-1}.

The hydroponic nutrient solution was made by combining equal parts (0.75 g·L^{-1} each) of 5N–5.2P–21.6K Jack's Professional Water-Soluble Fertilizer (J. R. Peter's) and 15.5N–0P–0K YaraLiva Calcinit (Yara International, Oslo, Norway) following the lettuce recipe by Mattson and Peters (2014). This nutrient recipe provided 150 mg·L^{-1} nitrogen (N), 39 mg·L^{-1} phosphorus (P), 162 mg·L^{-1} potassium (K), 139 mg·L^{-1} calcium (Ca), 47 mg·L^{-1} magnesium (Mg), 62 mg·L^{-1} sulfur (S), 2.3 mg·L^{-1} iron (Fe), 0.38 mg·L^{-1} manganese (Mn), 0.11 mg·L^{-1} zinc (Zn), 0.38 mg·L^{-1} boron (B), 0.113 mg·L^{-1} copper (Cu), and 0.075 mg·L^{-1} molybdenum (Mo). The pH of the nutrient solution was adjusted every 3 d to the range of 5.6–6.0, using 1 M potassium hydroxide (KOH) and 1 M sulfuric acid (H_2SO_4). After 1 week for plant establishment (7 d after transplanting into the hydroponic containers), the base nutrient solution was replaced, and five different concentrations of NaCl were added to the base nutrient solution (0, 0.05, 0.10, 0.20, and 0.40 M NaCl). Nine plants were randomly assigned to each NaCl treatment, and therefore

Fig. 2. Selected 4-week-old seedlings of ice plant just before transplant into hydroponic systems.

Fig. 3. Greenhouse setting for the hydroponic experiments with ice plant and NaCl concentration.

Table 1. Mean plant fresh weight, shoot fresh weight, and root fresh weight of ice plant in response to sodium chloride (NaCl) treatment in hydroponic nutrient solution over time (plants harvested 7, 14, or 21 d after treatment). Data represent means (± SE) of three experimental units times three replications over time.

Treatment (M NaCl)	Days after treatment			Significance across time
	7	14	21	
Plant fresh weight (g)				
0.00	84.1 ± 4.6 AB	257.9 ± 9.3 B	527.7 ± 19.6 C	L***
0.05	98.8 ± 7.2 A	355.2 ± 17.1 A	912.9 ± 38.3 A	L***
0.10	92.3 ± 7.8 A	282.3 ± 19.1 B	657.3 ± 22.4 B	L***
0.20	61.0 ± 4.4 B	236.5 ± 15.2 B	433.6 ± 22.7 C	L***
0.40	30.1 ± 6.7 C	65.5 ± 12.1 C	110.5 ± 16.3 D	L***
Shoot fresh weight (g)				
0.00	74.0 ± 4.0 AB	230.8 ± 7.4 B	453.8 ± 17.9 C	L***
0.05	89.5 ± 6.6 A	332.9 ± 16.6 A	877.1 ± 38.4 A	L***
0.10	83.8 ± 7.2 A	264.3 ± 18.2 B	627.1 ± 21.1 B	L***
0.20	55.6 ± 3.9 B	221.9 ± 14.6 B	409.0 ± 21.6 C	L***
0.40	27.4 ± 5.7 C	60.7 ± 11.3 C	103.6 ± 15.4 D	L***
Root fresh weight (g)				
0.00	10.1 ± 1.1 A	27.1 ± 2.7 A	73.9 ± 5.2 A	L***
0.05	9.3 ± 0.7 A	22.3 ± 1.3 AB	35.8 ± 2.0 B	L***
0.10	8.5 ± 0.6 AB	17.9 ± 1.2 BC	30.2 ± 1.8 B	L***
0.20	5.4 ± 0.6 BC	14.6 ± 0.9 C	24.7 ± 1.6 B	L***
0.40	2.7 ± 1.0 C	4.8 ± 0.9 D	7.0 ± 1.1 C	L***

Letters represent mean separation comparison across NaCl treatments within the same harvest day using Tukey's honestly significance difference ($\alpha = 0.05$).

Significance of linear (L) regression of a given treatment over treatment time. NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

a total of 45 plants were used for each crop cycle, with a total of three replicate crop cycles in the experiment. The nutrient solution plus corresponding NaCl was replaced every week (at day 14 and 21 after transplanting) to maintain electrical conductivity (EC). The EC was measured every 3 d and after each replacement of the nutrient solution and averaged 1.8 ± 0.1, 7.5 ± 0.1, 13.1 ± 0.3, 23.4 ± 0.3, and 42.3 ± 0.2 $dS \cdot m^{-1}$ (mean ± SD) for the 0, 0.05, 0.10, 0.20, and 0.40 M NaCl treatments, respectively.

Measurements. Three plants from each treatment (a total of 15 plants at each sample date) were harvested at day 7, 14, and 21 after NaCl treatment. At each harvest, the following measurements were taken: fresh weight (FW) and DW (following 72 h in an oven at 70 °C) of shoot (stem and leaf), leaf, root, and whole plant; number of leaves on main stem; and leaf surface area with a leaf surface area meter (LI-3100; LI-COR, Lincoln, NE). Shoots from the three plants per treatment per harvest date were pooled together for mineral nutrient tissue analysis at the Cornell Nutrient Analysis Laboratory (Ithaca, NY). EBCs were observed under ×16 magnification (field of view or field size is equal to 1.44 mm). A random plant was selected from each treatment. Three leaves were removed from each plant at separate locations (bottom, middle, and upper). These representative aliquots were sampled for qualitative and visual data. The experiment was replicated over time for a total of three times.

Experimental design and statistical analysis. The experiment was designed as a randomized complete block design. The above methods were replicated three times, and these crop cycles were treated as different blocks. Within each block, the experimental unit was one plant in a 4-L hydroponic container. Within each crop cycle, containers were randomly placed on the benches in the greenhouse. There were nine experimental units for each of the five NaCl treatments. Three experimental units from each NaCl treatment were randomly selected for destructive harvest at three time points (day 7, 14, and 21 after treatment). The data were analyzed using JMP 14.0 software (SAS Institute, Cary, NC). Analysis of variance and Tukey's honestly significance difference test were used to determine differences among NaCl treatments for each harvest date. Block effect was considered in the analysis. Data represent block centered means (± SE) which removed the block effect and more accurately reflected the difference among treatments.

Results

Fresh weight. In the first week of NaCl treatment, plants treated with lower NaCl concentrations (0.05 and 0.10 M) did not differ from the control, but plants treated with higher NaCl concentrations (0.20 and 0.40 M) started to show stunting effects of NaCl in terms of total FW, shoot FW, and root FW (Table 1). After 2 weeks of NaCl treatment, plants treated with 0.05 M NaCl exhibited the greatest shoot FW and therefore the greatest total FW. The control (0 M), 0.10, and 0.2 M NaCl groups had shoot FW smaller than the 0.05 M group but greater than the 0.40 M NaCl group. A similar pattern was observed at week 2 for total FW. Root FW for all treatments exhibited the pattern whereby increasing NaCl level in the nutrient solution decreased the plant root FW. After 3 weeks of NaCl treatment, the 0.05 M NaCl group exhibited greater shoot and total FW than the other groups, although it had only half of the root FW of the control. The 0.10 M NaCl group was ranked second and better than the control in terms of shoot and total FW. Similarly, it had much less root FW than the control but higher shoot FW, which resulted in higher total FW. In general, plants treated with lower NaCl concentrations (0.05 and 0.10 M NaCl) sacrificed some root weight but accumulated even more shoot weight. The 0.20 M NaCl group exhibited similar shoot and total FW as the control but lower root FW. The 0.40 M NaCl group exhibited the poorest result,

Table 2. Mean plant dry weight, shoot dry weight, and root dry weight and shoot/root ratio of ice plant in response to sodium chloride (NaCl) treatment in hydroponic nutrient solution over time (plants harvested 7, 14, or 21 d after treatment). Data represent means (± SE) of three experimental units times three replications over time.

Treatment (M NaCl)	Days after treatment 7	14	21	Significance across time
Plant dry weight (g)				
0.00	1.009 ± 0.057 AB	9.094 ± 0.286 A	19.567 ± 0.594 BC	L***
0.05	1.073 ± 0.054 A	10.162 ± 0.478 A	25.134 ± 1.203 A	L***
0.10	1.047 ± 0.072 A	9.188 ± 0.426 A	21.241 ± 0.931 AB	L***
0.20	0.777 ± 0.043 B	9.158 ± 0.550 A	16.590 ± 1.053 C	L***
0.40	0.417 ± 0.058 C	3.931 ± 0.221 B	6.587 ± 0.811 D	L***
Shoot dry weight (g)				
0.00	2.781 ± 0.146 A	7.649 ± 0.226 A	16.292 ± 0.565 BC	L***
0.05	3.162 ± 0.177 A	8.796 ± 0.418 A	22.245 ± 1.134 A	L***
0.10	3.215 ± 0.281 A	7.980 ± 0.396 A	18.758 ± 0.862 AB	L***
0.20	2.521 ± 0.123 AB	8.043 ± 0.503 A	14.792 ± 0.954 C	L***
0.40	1.796 ± 0.171 B	3.496 ± 0.173 B	5.951 ± 0.728 D	L***
Root dry weight (g)				
0.00	0.504 ± 0.029 AB	1.446 ± 0.082 A	3.275 ± 0.153 A	L***
0.05	0.536 ± 0.027 A	1.366 ± 0.066 AB	2.889 ± 0.142 AB	L***
0.10	0.524 ± 0.036 A	1.209 ± 0.046 AB	2.483 ± 0.091 B	L***
0.20	0.389 ± 0.021 B	1.115 ± 0.058 B	1.798 ± 0.115 C	L***
0.40	0.209 ± 0.029 C	0.434 ± 0.052 C	0.636 ± 0.088 D	L***
Shoot/root ratio				
0.00	5.558 ± 0.192 B	5.439 ± 0.173 C	5.013 ± 0.268 C	L*
0.05	5.806 ± 0.154 B	6.436 ± 0.222 BC	7.668 ± 0.434 B	L***
0.10	5.922 ± 0.208 B	6.538 ± 0.266 BC	7.474 ± 0.167 B	L***
0.20	6.432 ± 0.307 B	7.407 ± 0.337 AB	8.124 ± 0.239 AB	L**
0.40	9.363 ± 0.898 A	8.305 ± 0.405 A	9.395 ± 0.499 A	L^{NS}

Letters represent mean separation comparison across NaCl treatments within the same harvest day using Tukey's honestly significance difference ($\alpha = 0.05$).

Significance of linear (L) regression of a given treatment over treatment time. NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 3. Mean leaf water content and specific leaf area of ice plant in response to sodium chloride (NaCl) treatment in hydroponic nutrient solution over time (plants harvested 7, 14, or 21 d after treatment). Data represent means (± SE) of three experimental units times three replications over time.

Treatment (M NaCl)	Days after treatment 7	14	21	Significance across time
Leaf water content (%)				
0.00	96.3 ± 0.1 A	96.9 ± 0.0 B	96.8 ± 0.1 C	L*
0.05	96.6 ± 0.1 A	97.5 ± 0.0 A	97.8 ± 0.1 A	L***
0.10	96.3 ± 0.1 A	97.1 ± 0.1 B	97.3 ± 0.1 B	L***
0.20	95.6 ± 0.1 B	96.5 ± 0.1 C	96.7 ± 0.1 C	L***
0.40	93.5 ± 0.1 C	94.2 ± 0.1 D	94.5 ± 0.1 D	L***
Specific leaf area ($cm^2 \cdot g^{-1}$)				
0.00	177.9 ± 2.5 A	196.0 ± 1.9 A	197.4 ± 6.2 AB	L*
0.05	176.1 ± 3.2 A	211.4 ± 4.9 A	215.6 ± 7.6 A	L**
0.10	174.5 ± 3.7 A	180.3 ± 4.7 B	182.8 ± 5.2 B	L^{NS}
0.20	145.4 ± 3.2 B	148.8 ± 4.1 C	137.5 ± 3.5 C	L^{NS}
0.40	100.9 ± 2.0 C	92.9 ± 1.9 D	86.5 ± 6.6 D	L*

Letters represent mean separation comparison across NaCl treatments within the same harvest day using Tukey's honestly significance difference ($\alpha = 0.05$).

Significance of linear (L) regression of a given treatment over treatment time. NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

showing root FW only one-tenth that of the control. From the timeline perspective, all groups showed highly significant ($P < 0.001$) linear growth in total FW, shoot FW, and root FW (Table 1).

Dry weight. DW generally showed a similar trend as FW with a few notable differences. DW was less affected than FW by NaCl at lower concentrations (0–0.1 M). In other words, plants treated with 0.05 and 0.10 M NaCl accumulated water at a higher rate than accumulating dry mass. This was partially evidenced by the leaf water content result (Table 3). Second, the stimulating effect in terms of DW for the 0.05 M treatment did not appear until the last week. In other words, the outperformance of the 0.05 M NaCl group in terms of FW in the second week was mostly due to the accumulation of extra water. Third, the stunting effect of 0.40 M NaCl on DW was not as large as that on FW. This was also evidenced by the lowest leaf water content of this group (Table 3). Last, as NaCl level increased, the degree of decreased root DW was not as large as the degree of decreased root FW. In other words, when treated with higher NaCl levels, the reduction in water content was more dramatic than the reduction in dry matter content. From the timeline perspective, all plants showed highly significant ($P < 0.001$) linear growth in total, shoot, and root DW (Table 2).

Shoot/root ratio. Shoot/root ratio was higher with increased NaCl level. This trend gradually appeared from week 1 to week 3 (Table 2). However, the 0.40 M NaCl led to an increase in shoot/root ratio even by the first harvest due to poorer root performance than shoot performance. After the first week, both the control group and the 0.40 M NaCl group had relatively constant shoot/root ratio over time. In other words, they both gained shoot and root weights at the same rate, but this rate for the 0.40 M NaCl group was much lower than that for the control group. The other groups started with shoot/root ratios close to that of the control and over time had increased shoot/root ratios that were eventually close to that of the 0.40 M group. In other words, for these groups, the rate of accumulating shoot weight is higher than the rate of accumulating root weight.

Leaf number on the main stem, leaf fresh weight, leaf dry weight, and specific leaf area. Plants treated with higher NaCl concentrations developed fewer but thicker leaves (i.e., lower specific leaf area) (Tables 3 and 4). Visually, plants treated with higher NaCl concentrations had brighter green color (Fig. 4). Leaf FW showed a similar trend as shoot and total FW. The 0.05 M NaCl group started to stand out after 2 weeks of NaCl treatment and largely outperformed the control after 3 weeks of NaCl treatment. The 0.10 M NaCl group did not differ from the control group in the first 2 weeks of NaCl treatment but had greater leaf FW than control in the last. In terms of leaf FW and DW, the 0.40 M NaCl group was stunted in the first week of NaCl treatment, and this stunting effect became more obvious in the following weeks.

Fig. 4. Images of ice plants harvested after 21 d of sodium chloride (NaCl) treatment. Columns from left to right show 0, 0.05, 0.10 0.20, and 0.40 M NaCl treatments.

Table 4. Mean leaf number on the main stem, leaf fresh weight, and leaf dry weight of ice plant in response to sodium chloride (NaCl) treatment in hydroponic nutrient solution over time (plants harvested 7, 14, or 21 d after treatment). Data represent means (± SE) of three experimental units times three replications over time.

Treatment (M NaCl)	Days after treatment 7	14	21	Significance across time
Leaf number on the main stem				
0.00	15.0 ± 0.8 A	15.3 ± 0.2 A	18.0 ± 0.2 A	L^{NS}
0.05	15.3 ± 0.8 A	14.9 ± 0.3 AB	16.9 ± 0.1 B	L^{NS}
0.10	13.9 ± 0.7 A	13.8 ± 0.2 B	16.7 ± 0.1 B	L^{NS}
0.20	12.7 ± 0.4 AB	13.8 ± 0.2 B	15.7 ± 0.2 C	L**
0.40	10.4 ± 1.2 B	11.0 ± 0.3 C	14.7 ± 0.1 D	L***
Leaf fresh weight (g)				
0.00	71.8 ± 3.6 AB	212.8 ± 6.9 B	389.5 ± 14.3 C	L***
0.05	87.5 ± 6.4 A	312.7 ± 15.4 A	764.2 ± 31.3 A	L***
0.10	81.5 ± 6.8 A	247.6 ± 16.3 B	553.3 ± 17.1 B	L***
0.20	54.4 ± 3.7 B	209.6 ± 13.5 B	364.7 ± 19.2 C	L***
0.40	26.8 ± 5.2 C	58.9 ± 9.3 C	98.0 ± 11.0 D	L***
Leaf dry weight (g)				
0.00	2.639 ± 0.130 A	6.696 ± 0.182 A	12.597 ± 0.400 B	L***
0.05	3.031 ± 0.170 A	7.862 ± 0.371 A	17.297 ± 0.838 A	L***
0.10	3.066 ± 0.259 A	7.129 ± 0.318 A	14.866 ± 0.613 AB	L***
0.20	2.416 ± 0.117 AB	7.321 ± 0.448 A	12.262 ± 0.792 B	L***
0.40	1.735 ± 0.151 B	3.352 ± 0.100 B	5.518 ± 0.481 C	L***

Letters represent mean separation comparison across NaCl treatments within the same harvest day using Tukey's honestly significance difference ($\alpha = 0.05$).

Significance of linear (L) regression of a given treatment over treatment time. NS, *, **, ***Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Nutrient analysis: Epidermal bladder cells. Plants treated with NaCl accumulated much greater Na and Cl in the leaf tissue than control plants. There was also a pattern whereby total Na and Cl concentration increased even further as NaCl in nutrient solution increased from 0.05 to 0.40 M, although Na and Cl in 0.10 M and higher treatments were not statistically different from 0.05 M (Table 5). Plants containing more Na and Cl had a visually greater number of EBCs (Fig. 5). Although EBCs per unit area were not quantified, visually, within the same treatment, upper leaves that represented new growth had greater EBC density than middle and bottom leaves that represented older growth. The bottom leaves showed similar amounts of EBCs, indicating similar growing status before NaCl application.

The leaf concentration of macronutrients N and P was decreased by increased NaCl in hydroponic nutrient solution, whereas the leaf concentration of K remained relatively stable (Table 6). The intake of secondary macronu-

Table 5. Mean sodium (Na) and chloride (Cl) concentration of ice plant shoot tissue in response to sodium chloride (NaCl) treatment in hydroponic nutrient solution over time (plants harvested 7, 14, or 21 d after treatment). Data represents block centered means (± SE) of 1 pooled data of the 3 experimental units times 3 replications over time.

Treatment (M NaCl)	Days after treatment 7	14	21
Na concentration ($mg \cdot kg^{-1}$)			
0.00	27,590 ± 58,938 B	19,648 ± 72,670 B	13,558 ± 79,576 B
0.05	142,126 ± 16,969 AB	171,794 ± 5,588 AB	180,507 ± 15,382 AB
0.10	185,310 ± 1,688 AB	211,017 ± 1,716 AB	227,984 ± 13,055 AB
0.20	235,083 ± 28,466 A	272,047 ± 29,331 A	275,661 ± 33,666 A
0.40	262,634 ± 46,689 A	303,390 ± 47,430 A	305,206 ± 48,285 A
Cl concentration ($mg \cdot kg^{-1}$)			
0.00			12,991 ± 34,398 B
0.05			125,084 ± 4,428 A
0.10			137,824 ± 9,372 A
0.20			147,342 ± 11,012 A
0.40			167,755 ± 16,929 A

Letters represent mean separation comparison across NaCl treatments within the same harvest day using Tukey's honestly significance difference ($\alpha = 0.05$). Chloride concentration was measured only for the last harvest.

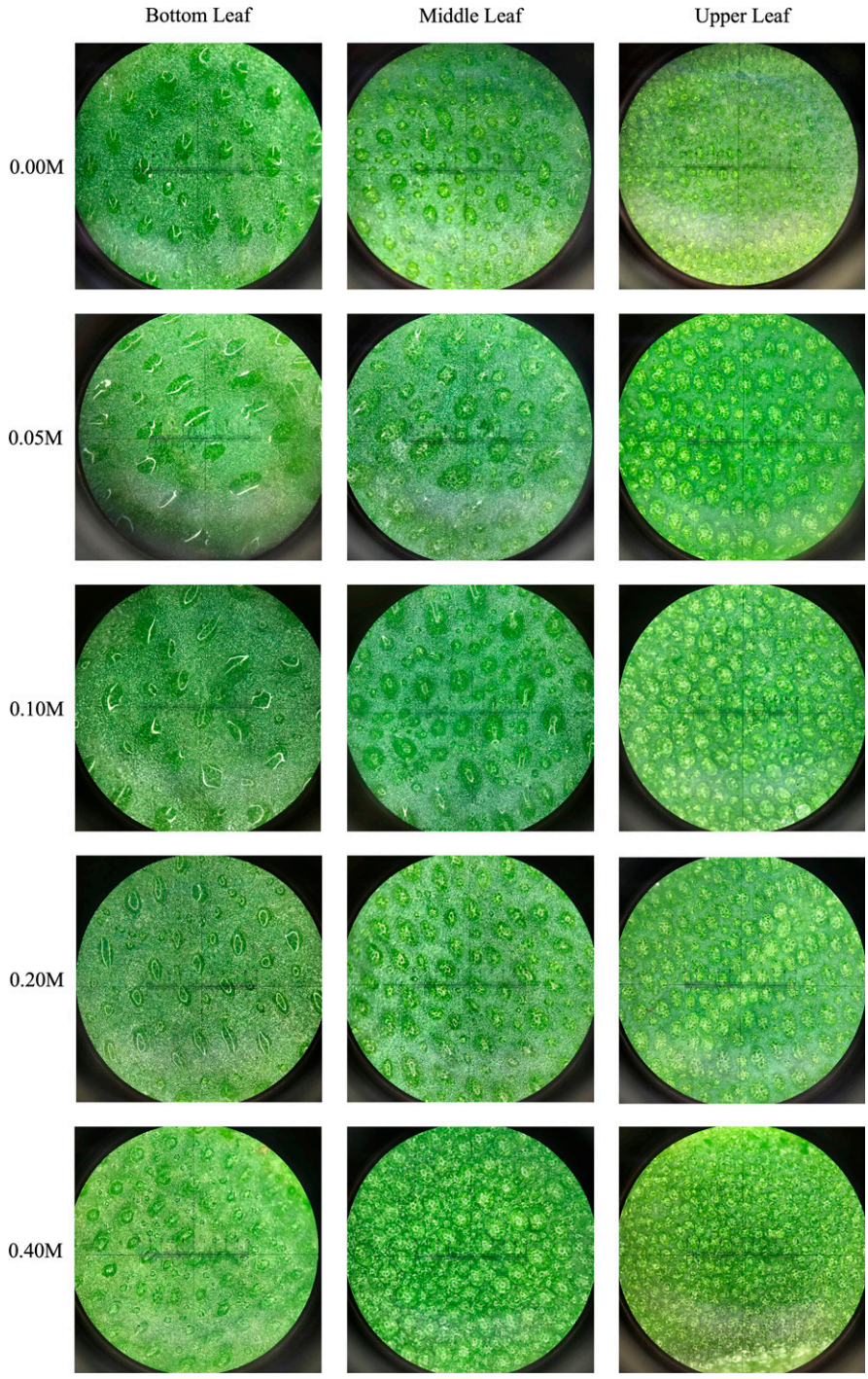

Fig. 5. Epidermal bladder cells (EBCs) on leaves of ice plants in response to NaCl treatment in hydroponic nutrient solution. Columns from left to right represent leaf samples from bottom, middle, and upper positions of the plants. Rows from top to bottom represent leaf samples of plants treated with 0, 0.05, 0.10, 0.20, and 0.40 M NaCl. The dissecting scope used ×16 magnification and the field of view (FOV) or field size is equal to 1.44 mm. Scale bars in the center of the pictures represent 5.88 mm.

trients Ca, Ma, and S was inhibited by increased NaCl in hydroponic nutrient solution (Table 7).

Discussion

Overall, optimum performance of ice plant (shoot and total FW/DW ratio) was observed at 0.05–0.10 M (Tables 1 and 2). Agarie et al. (2007) also found that elevated NaCl led to higher DW of ice plant; however, they reported optimum DW at a higher NaCl concentration of 0.10 and 0.20 M. This difference could be due to plant age of treatment/harvest. In this experiment plants were treated with NaCl 35 d after seeding, where in Agarie et al. (2007)'s experiment plants were treated at 45 d after seeding. Also, in this experiment root FW/DW was decreased when plants received increasing NaCl, whereas Agarie et al. (2007) reported only shoot DW results. Additionally, plants treated with 0.05 and 0.10 M NaCl distributed more energy to shoots than to roots, and the shoot/root ratio of these groups increased over time (Table 2). Previous research found that juvenile ice plants were less sensitive to salt stress because the amount of H^+-ATPase (V-ATPase), an enzyme known to respond to salt stress, did not change in the juvenile stage (Golldack and Dietz, 2001). Similarly, in this work, after the first week of NaCl treatments, plants receiving up to 0.2 M NaCl showed no negative effects of NaCl compared with control. However, Adams et al. (1998) concluded that ice plant was less adapted to salt stress at seedling or juvenile stage when organized tissues (such as well-developed EBCs) were not present yet. In the current study, whereas juvenile plants were less affected by lower salt treatments, the 0.4 M treatment group exhibited poor performance from the beginning. Additionally, EBCs, into which plants sequester NaCl, were present in juvenile plants, but they were empty and not filled with EBCs, as described by Adams et al. (1998). Similarly, the lack of EBC filling was observed in the current study in the first week of NaCl treatment (Fig. 5). Comparing the research results, when plants started to respond to NaCl treatment, a lower level of NaCl applied earlier brought a similar effect as a higher level of NaCl applied later. In the case of using ice plants as edible leafy greens, which requires earlier harvest, early application of low levels of NaCl might be more efficient (i.e., bringing the largest biomass with lower NaCl concentration).

Similar results were observed with a field production method. Atzori et al. (2017) mixed seawater into their irrigation water for ice plants and observed optimum growth at EC of 20 $dS \cdot m^{-1}$, and EC of 4, 8, 12, 16 $dS \cdot m^{-1}$ brought similar results of elevated biomass over control plants. In this research, 0.05, 0.10, 0.20 M NaCl treatments were measured to have EC of 7.5 ± 0.1, 13.1 ± 0.3, and 23.4 ± 0.3 $dS \cdot m^{-1}$ (mean ± SD), respectively. Therefore, the optimum growth in Atzori et al. (2017)'s research corresponds to 0.1–0.2 M NaCl concentration in the current study. Although the current study revealed that 0.05 M was optimum for growth, there were differences between the two studies. Seawater contains other elements besides NaCl, which resulted in a different nutrient composition from that of the current research. Also, the current project used hydroponics, resulting in stable EC and nutrient availability as compared with field conditions of Atzori et al. (2017). In general, the current study agrees with previous work that low levels of NaCl (0.05–0.20 M) promoted the growth of ice plant and that the optimum NaCl level varies depending on the growing conditions and time of NaCl application.

In this research, plants treated with 0.20 M NaCl were stunted in the first week of treatment but recovered in the following weeks, in terms of shoot and total FW. However, plants treated with 0.40 M NaCl were largely stunted at the beginning and had poorer performance over time (Tables 1 and 2). This indicates a possible upper bound of NaCl benefits in hydroponic production of around 0.20 M

Table 6. Mean nitrogen (N), phosphorus (P), and potassium (K) concentrations of ice plant shoot tissue in response to sodium chloride (NaCl) treatment in hydroponic nutrient solution over time (plants harvested 7, 14, or 21 d after treatment). Data represents block centered means (± SE) of 1 pooled data of the 3 experimental units times 3 replications over time.

Treatment (M NaCl)	Days after treatment		
	7	14	21
N concentration (%)			
0.00	65,437 ± 421 A	64,309 ± 277 A	57,953 ± 423 A
0.05	52,634 ± 1,182 B	48,542 ± 759 B	42,549 ± 1,788 B
0.10	51,237 ± 727 B	47,075 ± 154 B	43,218 ± 2,216 B
0.20	45,728 ± 824 C	42,105 ± 484 C	40,109 ± 421 B
0.40	32,333 ± 1,346 D	29,789 ± 144 D	31,778 ± 789 C
P concentration ($mg \cdot kg^{-1}$)			
0.00	11,561 ± 1,317 A	12,679 ± 1,158 A	14,741 ± 618 A
0.05	9,712 ± 72 A	10,250 ± 569 A	10,978 ± 231 B
0.10	9,746 ± 385 A	10,459 ± 246 A	9,868 ± 1,012 B
0.20	8,550 ± 242 AB	9,617 ± 383 AB	9,667 ± 119 B
0.40	4,708 ± 1,309 B	6,473 ± 301 B	8,228 ± 502 B
K concentration ($mg \cdot kg^{-1}$)			
0.00	26,226 ± 1,737 A	28,265 ± 2,197 A	27,468 ± 1,607 A
0.05	31,229 ± 1,429 A	31,424 ± 2,368 A	31,904 ± 1,600 A
0.10	30,356 ± 662 A	29,380 ± 1,651 A	27,748 ± 2,380 A
0.20	26,517 ± 1,038 A	24,227 ± 2,476 A	22,633 ± 2,942 A
0.40	26,784 ± 1,725 A	24,847 ± 2,806 A	23,336 ± 2,235 A

Letters represent mean separation comparison across NaCl treatments within the same harvest day using Tukey's honestly significance difference ($\alpha = 0.05$).

NaCl (i.e., NaCl concentration higher than this level had negative effects on the growth of ice plant). Other research showed a threshold of tolerance at 0.30 M NaCl (Herppich et al., 2012). Agarie et al. (2007) found a similar result to this study in that plants treated with 0.40 or 0.80 M NaCl were negatively affected to a large degree compared with the control. Agarie et al. (2007) compared performance of a mutant that did not develop proper EBCs as compared with wild-type plants. The wild-type performed better; for example, after 3 weeks of NaCl treatment it had a nearly 2-fold increase in DW compared with mutant plants. Therefore, EBCs played an important role in salt tolerance of ice plant, but this sequestration ability appears to reach a limit at 0.20 and 0.40 M NaCl. In the current study, there was an increased number of EBCs per unit leaf surface area (i.e., higher EBC density) with increased NaCl concentration in hydroponic nutrient solution (Fig. 5). However, with increasing NaCl plants developed fewer, smaller, and thicker leaves, which resulted in much less total surface area (Tables 3 and 4, Fig. 4). Compared with other halophytes, ice plant as an obligatory halophyte has a wide range of salt tolerance. For example, Grigore et al. (2012) tested whether halophytes required salt for their growth. They found halophytes such as golden samphire (*Inula crithmoides* L.), thick-leaved plantain (*Plantago crassifolia* Forssk), and costal medick (*Medicago marina* L.) grow better in salt-free soil than in saline soil. Salt is not required for their development but rather is a tool used to out-compete glycophytes in the saline environment (i.e., they are not competitive in the salt-free environment). Additionally, some dicotyledonous halophytes have optimal performance at 0.05–0.25 M NaCl (Flowers et al., 1986). Monocotyledonous halophytes generally have lower salt tolerance (< 0.05 M NaCl) (Glenn et al., 1999). Comparatively, the salt tolerance ability of ice plant is outstanding.

Table 7. Mean calcium (Ca), magnesium (Mg), and sulfur (S) concentrations of ice plant shoot tissue in response to sodium chloride (NaCl) treatment in hydroponic nutrient solution over time (plants harvested 7, 14, or 21 d after treatment). Data represents block centered means (± SE) of 1 pooled data of the 3 experimental units times 3 replications over time.

Treatment (M NaCl)	Days after treatment		
	7	14	21
Ca concentration ($mg \cdot kg^{-1}$)			
0.00	16,988 ± 883 A	20,051 ± 2,127 A	23,301 ± 2,452 A
0.05	10,493 ± 564 B	11,165 ± 408 B	12,851 ± 649 B
0.10	7,125 ± 407 C	6,868 ± 486 BC	7,349 ± 595 BC
0.20	5,581 ± 627 C	4,516 ± 769 C	4,043 ± 1,073 C
0.40	6,823 ± 324 C	4,210 ± 570 C	3,314 ± 910 C
Mg concentration ($mg \cdot kg^{-1}$)			
0.00	6,829 ± 407 A	8,160 ± 953 A	11,643 ± 216 A
0.05	4,090 ± 138 B	3,644 ± 272 B	3,701 ± 142 B
0.10	3,031 ± 138 BC	2,530 ± 203 B	2,346 ± 103 C
0.20	2,675 ± 180 C	2,066 ± 265 B	1,895 ± 145 C
0.40	2,993 ± 206 BC	2,171 ± 233 B	2,143 ± 118 C
S concentration ($mg \cdot kg^{-1}$)			
0.00	4,265 ± 214 A	4,639 ± 205 A	5,342 ± 274 A
0.05	3,018 ± 51 B	3,280 ± 46 B	4,064 ± 331 B
0.10	2,877 ± 47 B	2,896 ± 36 BC	3,004 ± 129 BC
0.20	2,587 ± 46 BC	2,700 ± 63 C	2,766 ± 144 C
0.40	2,105 ± 118 C	2,030 ± 76 D	2,116 ± 98 C

Letters represent mean separation comparison across NaCl treatments within the same harvest day using Tukey's honestly significance difference ($\alpha = 0.05$).

The world is addressing the salinization problem along two routes: desalinizing lands so that they can be arable for salt-sensitive crops and developing edible halophytes and halophyte-based agriculture (Hasanuzzaman et al., 2014; Ventura et al., 2015). Ice plant could serve both purposes as an edible, highly salt-tolerant halophyte. Compared with other hydroponic crops, ice plant is not damaged by higher NaCl concentrations in hydroponic nutrient solution but rather benefits from it. For example, hydroponic lettuce treated with a low level of NaCl (EC of standard nutrient solution plus low NaCl = 2.5 $dS \cdot m^{-1}$) lost about a quarter of its FW, and lettuce treated with a higher level of NaCl (EC = 3.7 $dS \cdot m^{-1}$) lost about half of its FW (Tas et al., 2005). However, in this research, 0.05, 0.10, 0.20 M NaCl treatments were measured to have EC of 7.5 ± 0.1, 13.1 ± 0.3, and 23.4 ± 0.3 $dS \cdot m^{-1}$ (mean ± SD), respectively, which were much higher than 2.5 or 3.7 $dS \cdot m^{-1}$. In this study the ability of ice plant to sequester high concentrations of NaCl was also quantified. Collectively these findings indicate that ice plant may be suitable for closed hydroponic production to mitigate NaCl accumulation. A dual ice plant–lettuce growing system may require less frequent replacement of hydroponic nutrient solution and therefore reduce waste and environmental pollution, and the ice plant would be an edible saleable crop. In addition to NaCl remediation of hydroponic solution, we speculate that ice plant may be valuable for the bioremediation of salinized soil. When consuming ice plant as an edible crop, it is important to avoid excessive intake of Na. The recommended daily Na consumption is 2,300 mg (National Academies of Sciences and Medicine, 2019). Based on FW, 100-g ice plant shoots grown with 0.05 M NaCl contained 397 mg Na and 100 g FW of ice plants treated 0.10 M NaCl contained 616 mg Na, which are both bearable.

Plants that had a higher tissue concentration of Na and Cl typically also had a lower concentration of other nutrients (on the basis of per kilogram DW). Similar results were observed by Agarie et al. (2007). Reduced root weight might affect the uptake of nutrients. However, with the expression of multiple genes, such as HAK/KUP (high affinity K^+ transporter/K^+ uptake transporter)-type genes and SKD1 (suppressor of K^+ transport growth defect), K uptake was less affected and K/Na ratio was maintained within certain range (Agarie et al., 2007). Additionally, NaCl treatment increased photosynthetically accumulated biomass, which is based mainly on carbon and water. According to Grigore et al. (2012), obligatory halophytes are specifically adapted to saline soils that are generally poor in nutrients, and they cannot survive in other habitats. The current research

showed poorer uptake of nutrients by ice plant with increased NaCl level in nutrient abundant fertilizing solution.

Conclusion

Overall, this study revealed that ice plant grown in hydroponics in a controlled environment benefited from 0.05 and 0.10 M NaCl additions to the hydroponic nutrient solution, making ice plant much more tolerant of NaCl than many other hydroponic crops. More work remains to be done on consumer preference for ice plant grown under different NaCl concentrations as well as potential sodium intake implications. The 0.05 M NaCl treatment may be optimum for plant yield and may cause less concern about salt intake than higher NaCl treatments. Adding 0.20 M or higher concentrations of NaCl is not recommended from both plant optimization and consumer Na intake perspectives.

The high NaCl tolerance of ice plant and accumulation in its leaves also indicates a strong potential of this plant as a source of bioremediation of saline soil and hydroponic water. Further research should be done to test ice plants incorporated into a hydroponic system to remediate NaCl accumulation as well as to provide another edible, saleable crop. For example, such experimentation could look at developing a dual lettuce–ice plant system whereby one tests only lettuce (no ice plant) at control and elevated NaCl concentrations as well as treatments with different fractions of lettuce–ice plant and varying NaCl concentrations. The ability of ice plant to remove NaCl from the nutrient solution over time and plant yield and quality would be assessed.

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