GROWTH, LEAF QUALITY AND EPIDERMAL BLADDER CELL SIZE OF ICE PLANT (MESEMBRYANTHEMUM CRYSTALLINUM L.) UNDER DIFFERENT CONCENTRATIONS OF NUTRIENT TREATMENT AND LED HEIGHT TREATMENT

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ABSTRACT

This study investigates the effect of nutrient treatment and LED height treatment on the growth, leaf quality and epidermal bladder cells size of ice plant (Mesembryanthemum crystallinum L.). The objectives of this study were to investigate the maximum growth, the ideal plant pigments quality and the maximum epidermal bladder cells size produced in the plants. Plant growths were maintained in an enclosed plant system – Farm Box under 13-h photoperiod of LED light using nutrient film technique systems. Environmental conditions such as air temperature, relative humidity and CO2 were controlled. Cultivation experiments were carried out in a control (EC = 0%) and four different nutrient concentration treatment (EC = 25%, 50%, 75% and 100%), under three different LED height treatment (mean light intensity 222, 213, 198 PPFD for 30cm, 35cm and 40cm height from planting tray) for 40 days of cultivation.

From the results of the plant growth among different nutrient concentration treatments, the 100% nutrient concentration showed the highest growth (leaf number, shoot number, plant height, leaf width, total fresh weight, leaf fresh weight, root fresh weight, stem fresh weight, total dry weight, leaf dry weight, root dry weight, stem dry weight and leaf area). The root length was the longest in 75% nutrient concentration (30cm LED height). At 100% nutrient concentration, the total fresh weight, leaf fresh weight and stem fresh weight were significantly ($P<0.05$) higher than all nutrient concentration in both 30cm and 35cm LED height. Among different LED height treatments, 30cm LED height has the highest growth (leaf number, shoot number, plant height, leaf width, leaf length, total fresh weight, leaf fresh weight, root fresh weight, stem fresh weight, total dry weight, leaf dry weight, root dry weight and stem dry weight) and was significantly ($P<0.05$) higher than 40cm LED height for total fresh weight, stem fresh weight, stem dry weight and leaf area (25% concentration nutrient).

In plant pigment, among different nutrient concentration treatments, plants cultivated using 50% nutrient concentration has the highest chlorophyll a, chlorophyll b and carotenoid in all LED height treatments. Chlorophyll a is significantly ($P<0.05$) higher than the 100% nutrient concentration (30cm LED height); chlorophyll b is significantly higher than the 25% nutrient concentration (all LED height); carotenoid is significantly higher than the 75% and 100% nutrient concentration (30cm and 35cm LED height).

In epidermal bladder cells size, among different nutrient concentration treatments, 75% nutrient concentration has largest EBC size on surface of abaxial leaf, adaxial leaf and stem. 75% nutrient concentration abaxial leaf's EBC (30cm LED height) and adaxial leaf's EBC (30cm and 35cm LED height) was significantly ($P<0.05$) larger than all other nutrient concentrations.

This study recommended the use of the 100% nutrient concentration and 30cm LED height treatment as the optimum growth condition for M. crystallinum, in the early planting stages (40 days) of M. crystallinum.

Keywords: Farm box, light intensity, plant pigment,

1.0 Introduction

By 2050, the total global population is estimated to reach 9.8 billion (United Nations, 2022) and the demand for food will increase. The controlled environmental agriculture that grows the vegetable crops, such as farm box or farm factory could become the new production system to meet those demands. *Mesembryanthemum crystallinum* L, is known as ice plant that is native to southern and eastern Africa, Western Asia and Southern Europe (Thompson, 2022). This species is stress-tolerant to drought and can switch from C_3 photosynthesis to Crassulacean Acid Metabolism (CAM). It is also tolerant to salt accumulation and able to grow in the salt marsh habitat. The leaves of *M. crystallinum* can be cooked or eaten raw as a pickle or spinach. It also has medicinal properties such as treating dysentery, liver and kidney, diseases relieving pain and itching (Thompson, 2022).

The nutrient concentration in hydroponics is important for the optimum growth of vegetable crops. Due to the salt tolerance of *M. crystallinum*, research in nutrient concentration using different salt concentrations was mostly investigated (He et al., 2021, Agarie et al., 2007, ~~Winter et al., 2005~~, Xia & Mattson, 2022). Few studies have investigated the optimum nutrient concentration formulation (Cha et al., 2016) for the growth of *M. crystallinum.*

Light is one of the important factors for the growth, reproduction and germination of plant crops (Li and Kubota, 2009). Recent studies investigated different red/blue LED luminaires for the growth of *M. crystallinum* (Kim et al., 2021, Weeplian & Ho, 2014, Weeplian et al., 2018, He et al., 2017). Investigation of the ideal light intensity for the growth of *M. crystallinum* was limited (Cha et al., 2014, Weeplian T & Ho Y-S. 2014, Xia & Mattson, 2022). Thus, the objective of this study was to investigate the effect on growth, leaf quality and epidermal bladder cell size of supplying different nutrient concentrations and LED height treatment.

1.1 Plant Materials and Culture Methods

The cultivation experiments were conducted between 1 June – 16 October 2020 in a controlled environment Farm Box located at Tunku Abdul Rahman University of Technology and Management, Johor Branch Campus, Segamat, Johor, Malaysia. The initial research was conducted between July – August 2019, with limited data and was excluded here.

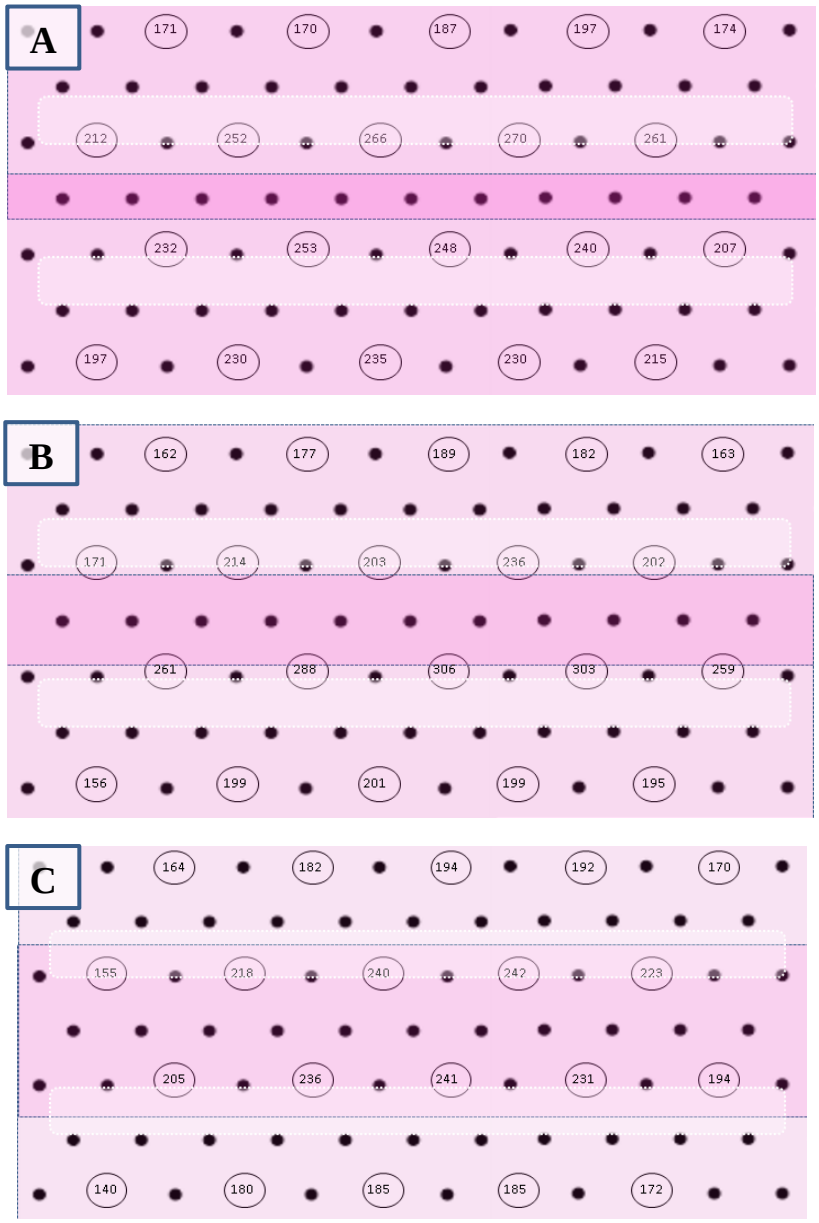
1.1.1 Germination

Seeds of *M. crystallinum* (Huayu Pai, China) were germinated in moist coco peat (Baba, Malaysia) and were transferred into polyurethane cubes for 10 days of incubation in a germination shelve under LED light with 50% nutrient concentration. The nutrient solution was flown for 15 minutes for every 20 minutes (day time) and 30 minutes (night times) from a 200L reservoir tank with a pump at a flow rate of 3.0 m^3h^{-1}.

1.1.2 Nutrient concentration treatment and LED height treatment

After 10 days of incubation, 20 seedlings were subsequently transplanted onto the planting tray for 30 days of cultivation. The plants were then cultured under a 13 hours photoperiod of light exposed under two rows of 1:4 blue:red LED ratios, 660:460 spectra, 40w, 200-240v, Sonneteck LED lights (Xiamen, China). Temperature and relative humidity in the growth room were maintained at 24°C and 70 – 85%. Plants were supplied with modified South China Agriculture University nutrient formula A (刘士哲, 2001) (KNO3 267mg/L, Ca(NO3)2.4H2O 472mg/L, MGSO4.7H2O 264automatically controlled mg/L. NH4NO3 53 mg/L, KH2PO4 100 mg/L, K2SO4 116 mg/L and micro nutrient Iron EDTA 15.4mg/L, H3B03 2.86mg/L, MnCl2.4h2O 1.8mg/L, ZnSO4.7H2O 0.22 mg/L, CuSO4.5H2O 0.078 mg/L and Na2MoO4.2H2O 0.025 mg/L). Cultivation experiments were carried out in 1) a control (EC = 0% negative control) and four different nutrient concentration treatments (EC = 25%, 50%, 75% and 100%) and 2) three different LED height treatments (30cm, 35cm and 40cm height from planting tray). The mean PPFD of 30cm, 35cm and 40cm height from planting tray were 222, 213, 198 PPFD, respectively. The plant spacing and light intensity distribution are showed in Figure 1. The light intensity was measured with TARGAS-1 Portable Photosynthesis System. The pH of the nutrient solution was maintained at 5.5 – 6.5. Cultivated plants were harvested after a total of 40 days of cultivation.

Figure 1: LED light distribution under three different LED height treatments (A= 30cm, B= 35cm and C= 40cm from planting tray) of 20 cultivated M. crystallinum. Middle red zone is the overlap light distribution area of two LED lights used in each treatment. Dotted white bars were the position of LED lights.



1.2 Plant Growth and Morphology

Plant morphological characters of leaf number, shoot number, plant height, leaf width and leaf length under different nutrient concentration treatment, and three LED height treatments were measured on 20 plants on day 10, 20, 30 and 40, respectively. Total fresh weight, leaf fresh weight, root fresh weight, stem fresh weight, total dry weight, leaf dry weight, root dry weight, stem dry weight, root length and leaf area under three LED height treatments in different nutrient concentration treatments were measured on 6 plants on day 40. The harvested plants for each treatment were carefully removed polyurethane cube from the roots. The shoot and root were blotted dry. The fresh weight and dry weight of the plant, leaves, stems and roots were weighed separately. The samples were wrapped individually in aluminum foil, dried at 65°C for 3 days. The leaf area was measured using the CI-202 Portable Laser Leaf Area Meter.

1.3 Light Microscope Analysis

The epidermal bladder cells (EBC) of the abaxial leaf, adaxial leaf and stem surface of day 40 cultivation plants were excised, observed and measured under a light microscope LEICA DM500 equipped with a LEICA ICC50 W camera. Ten EBC size of individual mature leaf were measured from three plants, respectively (the total number of samples per treatment was 30).

1.4 Measurement of Plant Pigment

The plant pigment extraction was based on a modified protocol from Atzori et al. (2017). The 2 cm x 2 cm of day 40 mature leaf sample was collected from four individual plants in three replicates (total number of samples per treatment was 12) and was kept at -80°C for 24 hours. Leaf samples were grinded and 0.1 g of leaf sample was mixed with 5 ml of ETOH in darkness at 4°C for 30 minutes. The leaf sample was centrifuged at 1000 rpm for 10 min. 3.5 ml of supernatant was collected and the absorbance was read at 665, 652 and 470 nm for chlorophyll a, chlorophyll b and carotenoid three times using a Jenway 7205 UV/Visible Spectophotometer.

1.5 Statistical analyses

Throughout the study, one-way ANOVA tests were performed on the obtained datasets. Post-hoc Tukey's tests were applied to each pair of groups. All statistical analyses were performed using Paleontological Statistics (PAST) version 4.01 (Hammer et al., 2001).

2.0 Results

2.1 Plant Growth (Leaf Number, Shoot Number, Plant Height, Leaf Width and Leaf Length)

0% nutrient concentration treatment as the negative control in this study was excluded for statistical analysis and discussion in all parameters. From day 10 to day 40 of cultivation (Table 1), the plant growths (leaf number, shoot number, plant height, leaf width and leaf length) were increased in all nutrient concentrations and LED height treatments. The highest plant growth was found in 100% nutrient concentration and 30cm LED height. On day 40 of cultivation, the highest leaf number (15.20 ± 1.51**)** was found in 100% nutrient concentration and was significantly higher than 25% and 50% nutrient concentration under 30cm LED height.

The shoot number was only appeared after day 30 of planting. The shoot number was increased from day 30 to day 40 in all nutrient concentrations and height treatments. On day 40 of cultivation, 100% nutrient concentration has the highest leaf number (9.70 ± 2.18**)**. The leaf number in 100% nutrient concentration was significantly higher than 25% and 50% nutrient concentration under all LED height (30cm, 35cm and 40cm height). The highest plant height (11.90 cm ± 3.10**)** was found in 100% nutrient concentration. Under 30cm LED height, the plant height was significantly higher in all nutrient concentration, under all LED heights. The leaf width was the widest (10.54 cm ± 1.72) in 100% nutrient concentration and was not significant with other nutrients concentrations and LED heights. The leaf length (17.07 cm ± 1.91**)** was the longest in 100% nutrient concentration and was significantly higher than 25% and 50% nutrient concentration under 30cm and 35cm LED height.

Table 1: *Mesembryanthemum crystallinum* leaf number, shoot number, plant height, leaf width and leaf length in different nutrient concentration under the three LED height treatments in four growing periods.

Day	Nutrient concentration (%)	Leaf number (n=20)			Shoot number (n=20)			Plant height (n=20)			Leaf Width (n=20)			Leaf Length (n=20)		
		40cm	35cm	30cm	40cm	35cm	30cm	40cm	35cm	30cm	40cm	35cm	30cm	40cm	35cm	30cm
10	**0**	4.00± 0.00	4.00± 0.00	4.00± 0.00	-	-	-	0.05 ± 0.07	0.04± 0.06	0.06± 0.07	0.25 ± 0.04	0.24 ± 0.06	0.24 ± 0.07	0.39 ± 0.10	0.36± 0.11	0.50± 0.11
	25	3.90± 0.45	3.90± 0.45	4.00± 0.00	-	-	-	0.68 ± 0.82	0.69± 1.27	0.42± 0.42	0.38 ± 0.41	0.39 ± 0.40	0.41 ± 0.50	1.00 ± 1.21	0.73 ± 0.64	0.72± 0.61
	50	3.70± 0.73	3.50± 0.89	3.60± 0.82	-	-	-	0.16 ± 0.24	0.12± 0.13	0.19± 0.20	0.25 ± 0.09	0.26 ± 0.09	0.27 ± 0.08	0.49 ± 0.20	0.42± 0.20	0.40± 0.16
	75	3.90± 0.45	3.80± 0.62	3.75± 0.64	-	-	-	0.20 ± 0.25	0.14± 0.13	0.17± 0.26	0.31 ± 0.12	0.29 ± 0.11	0.27 ± 0.09	0.54 ± 0.30	0.45± 0.27	0.42± 0.26
	100	4.00± 0.00	3.90± 0.45	4.00± 0.00	-	-	-	0.40 ± 0.21	0.30± 0.20	0.30± 0.31	0.30 ± 0.14	0.30 ± 0.09	0.30 ± 0.05	0.60 ± 0.24	0.50± 0.33	0.50± 0.25
20	**0**	5.10± 1.02	4.90± 1.02	5.80± 0.62	-	-	-	0.34 ± 0.23	0.23± 0.17	0.33± 0.21	0.39 ± 0.20	0.33 ± 0.11	0.41 ± 0.15	0.89 ± 0.55	0.67± 0.31	0.98± 0.47

	25	7.40± 0.94	7.30± 0.98	7.50± 0.89	-	-	-	1.14 ±0.24	0.90± 0.16	1.45± 0.33	1.06 ± 0.38	1.50± 0.22	1.37 ± 0.68	2.40 ± 0.70	2.90± 0.43	2.44± 0.97
	50	7.40± 0.94	7.60± 0.82	7.80± 0.62	-	-	-	1.22 ± 0.35	1.29± 0.41	1.51± 0.58	1.25 ± 0.5**a**	1.70 ± 0.88 **ab**	1.91 ± 0.93 **b**	2.52 ± 0.95 **a**	3.34± 1.36**ab**	3.60± 1.47**b**
	75	7.60± 1.05	7.20± 1.88	7.20± 1.01	-	-	-	1.34 ± 0.50	1.36± 0.54	1.30± 0.38	2.92 ± 1.22 **b**	1.77 ± 1.10 **b**	1.56 ± 0.91 **a**	2.20 ± 1.09 **ab**	3.37± 1.39**a**	3.08± 1.15**b**
	100	7.90± 0.45	7.80± 0.62	7.70± 0.73	-	-	-	1.40 ± 0.48	1.68± 0.47	1.58± 0.56	1.82 ± 0.86	2.07 ± 1.07	2.05 ± 0.96	3.30 ± 1.28	3.55± 1.22	3.54± 1.11
30	**0**	6.70± 1.17	6.80± 1.20	7.50± 0.89	-	-	-	0.73 ± 0.47	0.53± 0.33	0.77± 0.39	0.62 ± 0.36	0.51 ± 0.21	0.66 ± 0.24	1.13 ± 0.63	0.97± 0.46	1.31± 0.65
	25	9.80± 2.42**A**	10.20± 2.67	10.74 ± 3.37	1.10± 1.52**A**	1.40 ± 1.73 **AB**	1.89 ± 1.70	2.40 ± 0.61 **A**	2.49± 0.66**A**	2.64± 0.79**A**	3.77 ± 1.17 **aA**	4.35 ± 1.36 **abA**	4.92 ± 1.86 **bA**	6.35 ± 1.53 **BC**	6.53± 1.37**A**	6.99± 2.43**A**
	50	9.90± 1.02**B**	10.30 ± 1.17	10.40 ± 1.39	2.50± 1.70**A bc**	2.20 ± 1.70 **bB**	0.90 ± 1.37 **b**	2.65 ± 0.55 **AB**	3.24± 1.19**A B**	3.24± 0.87**A B**	4.41 ± 1.48 **aA B**	5.57 ± 2.45 **abA B**	6.13 ± 2.41 **bA B**	6.89 ± 1.59 **BC**	8.23± 2.74**A B**	8.45± 2.16**A B**
	75	10.60 ± 1.31**A B**	10.60± 1.31	10.80 ± 1.20	3.20± 1.50**1C**	2.60 ± 2.06 **AB**	1.85 ± 2.11	3.29 ± 1.04 **B**	3.79± 1.28**B**	3.53± 0.94**B**	5.84 ± 2.37 **B**	6.38 ± 2.29 **B**	6.61 ± 2.17 **B**	8.96 ± 3.18 **A**	9.43± 2.78**A**	9.77± 2.68**B**
	100	11.60 ± 1.79**A**	11.85± 2.87	12.20 ± 2.82	1.70± 1.63**A B**	2.45 ± 2.16 **B**	2.70 ± 2.08	2.83 ± 1.02 **AB**	3.20± 0.87**A B**	3.18± 0.76**A B**	4.91 ± 1.83 **AB**	5.82 ± 2.31 **AB**	6.15 ± 2.23 **AB**	7.84 ± 1.91 **AB C**	8.32± 2.48**A B**	8.35± 2.07**A B**
40	**0**	8.00± 1.45	7.60± 1.79	8.30± 1.17	-	-	-	0.92 ± 0.44	0.79± 0.45	1.20± 0.53	0.83 ± 0.49	0.72 ± 0.31	0.86 ± 0.33	1.51 ± 0.86	1.32± 0.61	1.55± 0.70
	25	12.85 ± 3.01**a BC**	13.40± 3.14**bc AB**	13.95 ± 3.19**c AB**	6.20± 2.10**aA B**	6.80 ± 1.93 **abA B**	7.74 ± 2.46 **bcA B**	6.12 ± 1.72 **Ba**	6.71± 1.76**b B**	7.74± 2.46**a C**	8.18 ± 2.09 **aA B**	8.47 ± 2.11 **bcA B**	9.34 ± 3.04 **c**	13.29± 3.13**a A**	13.77± 3.27**ab AB**	14.49 ± 4.62**b AB**
	50	12.20 ± 1.28**a BC**	13.10± 1.65**ab B**	13.40 ±1.73 **bC**	4.25± 1.83**aC**	5.70 ± 2.77 **abB**	6.40 ± 2.48 **bC**	4.64 ± 0.56 **aB**	5.64± 1.54**b B**	6.93± 2.12**b B**	7.29 ± 1.50 **aB**	8.13 ± 2.29 **abB**	9.09 ± 2.04 **b**	10.74± 1.42**aB**	12.34± 3.01**ab C**	13.33 ± 2.37**b C**
	75	13.30 ± 1.98**A**	13.60± 1.90**A B**	14.21 ± 3.49**B C**	6.30± 3.20**B**	6.90 ± 2.63 **B**	7.68 ± 2.62 **BC**	6.20 ± 2.31 **B**	6.72± 1.93**B**	7.29± 2.61**B C**	8.62 ± 2.57 **bA B**	9.47 ± 2.03 **abA B**	10.15± 3.31 **a**	13.35± 2.50**abA**	13.95± 2.88**aB**	14.74 ± 4.30**b BC**
	100	14.40 ± 1.05**A**	14.80± 1.01**A**	15.20 ± 1.51**A**	8.50± 2.33**A**	9.30 ± 2.08 **A**	9.70 ± 2.18 **A**	10.07± 2.74**A**	11.69 ± 3.26**A**	11.90 ± 3.10**A**	9.88 ± 2.35 **A**	10.50± 2.47**A**	10.54± 1.72	14.06± 2.72**aA**	16.78± 1.29**b A**	17.07 ± 1.91**b A**

Different lowercase letters in a column indicate a significant difference among three LED height treatments according to Tukey's post-hoc test ($P < 0.05$).

Different uppercase letters in a row indicate a significant difference among four different nutrient concentration treatment according to Tukey's post-hoc test ($P < 0.05$). 0% nutrient concentration as negative control is excluded in statistical analyses.

30, 35 and 40 indicate LED height is 30cm, 35cm and 40cm from the plants, respectively

2.2 Plant Growth (Total Fresh Weight, Leaf Fresh Weight, Root Fresh Weight, Stem Fresh Weight, Total Dry Weight, Leaf Dry Weight, Root Dry Weight, Stem Dry Weight, Root Length and Leaf Area)

The 100% nutrient concentration showed the highest growth in all parameters (total fresh weight, leaf fresh weight, root fresh weight, stem fresh weight, total dry weight, leaf dry weight, root dry weight, stem dry weight and leaf area) except the root length (50.95 cm ± 5.19) was the longest in 75% nutrient concentration, under 30cm LED height (Table 2). In 100% nutrient concentration, the total fresh weight, leaf fresh weight and stem fresh weight were significantly higher than all nutrient concentration in 30cm and 35cm LED height, and was significantly higher than 25% and 50% nutrient concentration in 40cm LED height. The total dry weight and stem dry weight were significantly higher than 25% and 50% nutrient concentration (30cm and 35cm LED height). The leaf area was significantly higher than 25% nutrient concentration in 30cm LED height, and significantly higher than 25% and 50% nutrient concentration in both 35cm and 40cm LED height.

Among different LED height treatments, the 30cm LED height has the highest growth in total fresh weight, leaf fresh weight, root fresh weight, stem fresh weight, total dry weight, leaf dry weight, root dry weight and stem dry weight. The 30cm LED height is significantly higher than 40cm LED height in total fresh weight, stem fresh weight, stem dry weight and leaf area (25% concentration nutrient). The 30cm LED height was also significantly higher than all height treatments in leaf fresh weight (50% concentration nutrient) and leaf dry weight (100% concentration nutrient). Kim et al. (2018) found higher light intensity (15 PPFD) yield higher growth of M. crystallinum. Weeplian & Ho (2014) also found the growth and leaf area of M. crystallinum was significantly larger at high intensity (190 PPFD). In this study, the 30cm LED height treatments that yield the highest growth was mean 222 PPFD. This light intensity is suggested as the optimum light for the early growing stage of M. crystallinum in the hydroponic industry.

Table 2: Mesembryanthemum crystallinum total fresh weight, leaf fresh weight, root fresh weight, stem fresh weight, total dry weight, leaf dry weight, root dry weight, stem dry weight, root length and leaf area under the three LED height treatments in four nutrient concentration treatments.

Parameter	LED height treatment (cm)	Concentration nutrient				
		0%	25%	50%	75%	100%
Total fresh weight (g)(n=6)	30	0.59 ± 0.44	158.30 ± 38.38**aA**	137.03 ± 20.56**aA**	150.52 ± 34.80**A**	239.42 ± 48.01**aB**
	35	1.40 ± 0.36	130.87 ± 40.60**abA**	126.92 ± 10.90**abA**	139.37 ± 43.40**A**	216.34 ± 61.11**abB**
	40	2.02 ± 0.92	101.02 ± 23.60**bA**	95.24 ± 32.28**bA**	141.39 ± 51.03**AB**	176.25 ± 46.42**bB**
Leaf fresh weight (g)(n=6)	30	0.18 ± 0.06	133.03 ± 30.96**aA**	120.09 ± 17.15**aA**	129.72 ± 30.30**A**	188.82 ± 37.93**B**
	35	0.82 ± 0.27	110.26 ± 35.45**abA**	111.54 ± 9.59**abA**	120.49 ± 37.06**A**	177.80 ± 48.90**B**
	40	1.48 ± 0.90	85.07 ± 19.30**bAC**	79.38 ± 25.26**bC**	122.23 ± 44.43**ABC**	146.35 ± 37.24**B**
Root fresh weight (g)(n=6)	30	0.18 ± 0.06	9.76 ± 2.17**aA**	6.84 ± 1.94**aBC**	8.50 ± 1.84**AC**	11.85 ± 5.13**A**
	35	0.29 ± 0.32	9.49 ± 1.84**abA**	6.36 ± 1.71**abBC**	6.63 ± 1.53**BCD**	10.55 ± 2.73**AD**
	40	0.24 ± 0.14	8.12 ± 2.61**bABC**	7.40 ± 2.45**bBC**	7.35 ± 1.47**C**	11.67 ± 3.16**A**
Stem fresh weight (g)(n=6)	30	0.24 ± 0.36	15.52 ± 5.87**aA**	10.10 ± 1.81**A**	12.29 ± 4.58**A**	38.75 ± 19.71**aB**
	35	0.29 ± 0.26	11.12 ± 4.10**abA**	9.02 ±1.12**A**	12.26 ± 5.43**A**	27.99 ± 11.13**abB**
	40	0.29 ± 0.31	7.83 ± 2.58**aA**	8.458 ± 4.64**A**	11.81 ± 7.00**AB**	18.23 ± 8.56**bB**
Total dry weight (g)(n=6)	30	0.12 ± 0.03	3.11 ± 0.60**A**	3.28 ± 0.53**A**	4.01 ± 0.91**AB**	5.30 ± 1.36**abB**
	35	0.08 ± 0.02	2.98 ± 0.61**A**	3.59 ± 0.69**AB**	3.24 ± 0.68**A**	5.27 ± 1.54**aB**
	40	0.12 ± 0.07	2.50 ± 0.57**A**	3.22 ± 0.79**AB**	3.35 ± 0.67**B**	3.62 ± 0.76**bB**
Leaf Dry weight (g)(n=6)	30	0.10 ± 0.02	2.03 ± 0.43**A**	2.38 ± 0.41**AB**	2.93 ± 0.67**ABC**	3.64 ± 0.85**abC**
	35	0.05 ± 0.02	2.11 ± 0.39**B**	2.72 ± 0.64**B**	2.25 ± 0.50**B**	3.60 ± 0.88**aA**
	40	0.10 ± 0.07	1.83 ± 0.41**A**	2.37 ± 0.57**AB**	2.36 ± 0.41**AB**	2.45 ± 0.48**bB**
Root dry weight (g)(n=6)	30	0.01 ± 0.01	0.49 ± 0.08	0.51 ± 0.18	0.58 ± 0.11	0.60 ± 0.24
	35	0.01 ± 0.01	0.48 ± 0.50	0.49 ± 0.11	0.51 ± 0.12	0.53 ± 0.14
	40	0.02 ±0.01	0.40 ± 0.09**A**	0.43 ± 0.14**AB**	0.54 ± 0.05**B**	0.56 ± 0.14**B**
Stem dry weight (g)(n=6)	30	0.01 ± 0.01	0.60 ± 0.25**aA**	0.38 ± 0.04**A**	0.50 ±0.18**A**	1.06 ± 0.32**B**
	35	0.02 ± 0.01	0.40 ± 0.15**abA**	0.38 ± 0.06**A**	0.48 ± 0.20**A**	1.14 ± 0.60**B**
	40	0.01 ± 0.01	0.27 ± 0.10**bA**	0.42 ± 0.21**AB**	0.45 ± 0.22**AB**	0.61 ± 0.30**B**
Root length (cm)(n=6)	30	16.78 ± 3.05	42.17 ± 4.72**abA**	43.75 ± 3.55**A**	50.95 ± 5.19**BC**	49.33 ± 9.67**AC**
	35	16.15 ± 3.72	48.67 ± 8.64**a**	46.33 ± 9.85	48.92 ± 11.77	58.17 ± 13.88
	40	18.28 ± 6.38	38.33 ± 6.32**bA**	42.33 ± 8.90**A**	46.42 ± 5.63**AB**	55.17 ± 7.41**B**
Leaf area (cm^2) (n=6)	30	9.99 ± 2.29	914.74 ± 233.69**aABC**	809.04 ± 117.18**aBC**	838.59 ± 365.61**C**	1234.63 ± 19.22**A**
	35	6.75 ± 1.98	720.15 ± 175.58**abA**	735.84 ± 82.32**abA**	867.42± 256.38**AB**	1187.39 ± 35.78**B**
	40	11.74 ± 6.03	589.13 ± 133.66**bA**	586.33 ± 155.56**bA**	887.40 ± 229.19**AB**	987.10 ± 219.53**B**

Different lowercase letters in a column indicate a significant difference among three LED height treatments according to Tukey's post-hoc test ($P < 0.05$).

Different uppercase letters in a row indicate a significant difference among four different nutrient concentration treatment according to Tukey's post-hoc test ($P < 0.05$). 0% nutrient concentration as negative control is excluded in statistical analyses.

30, 35 and 40 indicate LED height is 30cm, 35cm and 40cm from plants, respectively.

2.3 Chlorophyll a, chlorophyll b and carotenoid.

The synthesis of chlorophyll in plants depend on several factors such as light intensity, nutrient, temperature, plant genetic, amount of water, oxygen and carbohydrates (Fevria et al., 2023). In this study, 50% nutrient concentration has the highest chlorophyll a, chlorophyll b and carotenoid (Table 3) in all different LED height treatments. Nutrient concentration treatment ought to be the main factor for the high chlorophyll a, chlorophyll b and carotenoid concentration in the plants than the LED height treatment. The chlorophyll a in 50% nutrient concentration was range 0.71 – 0.85, and was significantly higher than 100% nutrient concentration (30cm LED height treatment). The chlorophyll b in 50% nutrient concentration was range 0.41 – 0.48, and was significantly higher than 25% nutrient concentration (all LED height treatments). The carotenoid in 50% nutrient concentration was range 0.76 – 0.87 and was significantly higher than 75% and 100% nutrient concentration (30cm and 35cm LED height treatments). Chlorophyll plays an important role in plant photosynthesis. Chlorophyll helps the plants to absorb and convert the light energy into the chemical energy (Woodward et al., 1960). The higher the chlorophyll content, the higher the growth and development process in plants. Besides, chlorophyll also provides many health benefits such as promoting anticancer properties, detoxification and as an antioxidant to people. Although the 50% nutrient concentration plants in this study has lower plant growth rate (Table 2) than 100% nutrient concentration plants, yet the high chlorophyll concentration in 50% nutrient concentration plants may indicates a healthier plants for people.

Table 3: Mesembryanthemum crystallinum chlorophyll a, chlorophyll b and carotenoid under the three LED height treatments in five nutrient concentration treatments.

Nutrient concentration		**0%**	**25%**	**50%**	**75%**	**100%**
Chlorophyll a	**30cm**	0.19 ± 0.04	0.63 ± 0.14**C**	0.78 ± 0.05**BC**	0.39 ± 0.12**AB**	0.45 ± 0.05**A**
	35cm	0.21 ± 0.05	0.62 ± 0.11**A**	0.85 ± 0.09**AB**	0.34 ± 0.09**B**	0.52 ± 0.05**B**
	40cm	0.21 ± 0.03	0.59 ± 0.05**A**	0.71 ± 0.24**AB**	0.49 ± 0.10**B**	0.54 ± 0.15**B**
Chlorophyll b	**30cm**	0.12 ± 0.03	0.37 ± 0.08**A**	0.46 ± 0.04**BC**	0.22 ± 0.06**B**	0.25 ± 0.03**B**
	35cm	0.12 ± 0.03	0.37 ± 0.06**A**	0.48 ± 0.05**BC**	0.22 ± 0.04**B**	0.30 ± 0.03**B**
	40cm	0.13 ± 0.02	0.36 ± 0.03**A**	0.41 ± 0.13**B**	0.28 ± 0.06**BC**	0.32 ± 0.07**D**
Carotenoid	**30cm**	0.24 ± 0.02	0.68 ± 0.13**A**	0.86 ± 0.05**bA**	0.43 ± 0.10**B**	0.54 ± 0.07**B**
	35cm	0.29 ± 0.05	0.69 ± 0.09**A**	0.87 ± 0.07**bA**	0.44 ± 0.07**B**	0.58 ± 0.05**B**
	40cm	0.31 ± 0.07	0.67 ± 0.05	0.76 ± 0.21**a**	0.56 ± 0.09	0.86 ± 0.05

Different lowercase letters in a column indicate a significant difference among three LED height treatments according to Tukey's post-hoc test ($P < 0.05$).

Different uppercase letters in a row indicate a significant difference among four different nutrient concentration treatment according to Tukey's post-hoc test ($P < 0.05$). 0% nutrient concentration as negative control is excluded in statistical analyses.

30, 35 and 40 indicate LED height is 30cm, 35cm and 40cm from the plants, respectively.

2.4 Epidermal bladder cells

Among different nutrient concentration treatments, the 75% nutrient concentration has largest epidermal bladder cells' (EBC) size on surface of abaxial leaf, adaxial leaf and the stem (Table 7, Figure 2). In 75% nutrient concentration, the abaxial leaf EBCs' width (30cm LED height) and length (all LED heights) was significantly larger than all nutrient concentrations. The width and length of adaxial leaf EBCs' were significantly higher than all nutrient concentrations in 30cm and 35cm LED height. The adaxial leaf EBCs' width (30cm and 35 LED heights) and length (30cm LED height) was significantly larger than all nutrient concentrations. Among the different LED height treatments, the largest size of EBC was found inconsistent between different LED heights. However in 30cm LED height, the length of the stem's EBC was significantly larger than other LED heights (25% and 50% nutrient concentration).

Besides optimizing the plant growth, it is important to understand the size of EBCs in relation to sensory acceptance of M. crystallinum. It has been suggested that EBCs able to store water and salt (Na+) (Agarie et al., 2007) in the plant. Epidermal bladder cells also act as secondary epidermis that reduces water loss through respiration (Agarie et al., 2007). To date, most studies investigated the salt tolerant properties of EBCs in M. crystallinum (Agarie et al., 2007, He et al., 2021, Moog et al., 2022, Xia & Mattson, 2022). Xia et al (2022) found that different salt tolerant (0 – 0.4 M NaCl) of M. crystallinum had the right crunchiness. The high salt concentration (0.4 M NaCl) of M. crystallinum was reported not juicy. In this study, the 75% nutrient concentration recorded the largest EBCs size in M. crystallinum. This may indicate better juiciness and crunchiness texture of M. crystallinum for the consumers. Further study in to sensory acceptance of M. crystallinum is required.

Figure 2: A. Adaxial leaf follicle. B. Abaxial leaf follicle. C. Stem follicle. D. Adaxial leaf. E. Abaxial leaf. F. Stem. A-C under 30cm LED height treatment, 75% nutrient concentration.

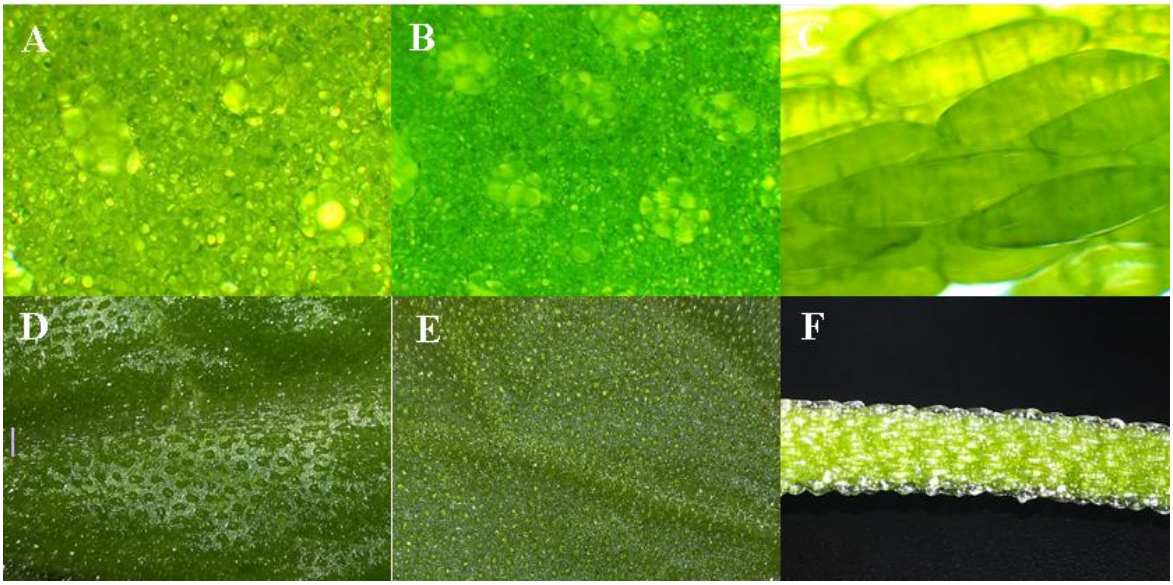


Table 4: Mesembryanthemum crystallinum epidermal bladders cells (EBCs) size from abaxial leaf, adaxial leaf and stem surface under the three LED height treatments in four nutrient concentration treatments.

			Nutrient concentration				
	Parameter	**LED Height**	**0%**	**25%**	**50%**	**75%**	**100%**
Abaxial leaf (n=30)	Width	30	0.51 ± 0.07	0.68 ± 0.07**bA**	0.95 ± 0.13**aC**	1.14 ± 0.16**aB**	0.70 ± 0.10**aA**
		35	0.56 ± 0.05	0.68 ± 0.10**bA**	0.72 ± 0.11**bA**	0.82 ± 0.24**bB**	0.76 ± 0.06**bAB**
		40	0.53 ± 0.11	0.77 ± 0.11**aAC**	0.65 ± 0.08**cB**	0.84 ± 0.20**bC**	0.71 ± 0.13**abAB**
	Length	30	0.63 ± 0.08	0.93 ± 0.13**bA**	1.20 ± 0.14**aC**	1.46 ± 0.20**bB**	0.94 ± 0.16**A**
		35	0.73 ± 0.10	0.93 ± 0.12**bA**	0.96 ± 0.13**bA**	1.44 ± 0.43**bB**	0.92 ± 0.15**A**
		40	0.77 ± 0.10	1.03 ± 0.15**aA**	0.86 ± 0.13**cB**	1.18 ± 0.22**aC**	0.89 ± 0.09**B**
Adaxial leaf (n=30)	Width	30	0.48 ± 0.12	0.52 ± 0.11**A**	**0.60 ± 0.08aA**	0.83 ± 0.25**bB**	**0.53 ± 0.12bC**
		35	0.43 ± 0.05	0.53 ± 0.06**A**	0.50 ± 0.07**bA**	0.78 ± 0.22**bB**	0.52 ± 0.04**bA**
		40	0.50 ± 0.12	**0.53 ± 0.14A**	0.51 ± 0.05**bAB**	0.57 ± 0.08**aA**	0.47 ± 0.07**aB**
	Length	30	0.64 ± 0.13	0.82 ± 0.11**A**	0.97 ± 0.22**aB**	1.13 ± 0.28**bC**	0.89 ± 0.11**aAB**
		35	0.74 ± 0.10	0.85 ± 0.14**A**	0.78 ± 0.09**bA**	0.99 ± 0.27**bB**	0.79 ± 0.14**bA**
		40	0.76 ± 0.15	0.90 ± 0.15**A**	0.69 ± 0.10**bB**	0.79 ± 0.13**aC**	0.74 ± 0.16**bBC**
Stem (n=30)	Width	30	-	0.49 ± 0.08**A**	0.51 ± 0.05**A**	0.55 ± 0.06**bB**	0.49 ± 0.04**bA**
		35	-	0.48 ± 0.05**A**	0.50 ± 0.05**A**	0.64 ± 0.19**aB**	0.50 ± 0.04**bA**
		40	-	0.46 ± 0.04**A**	0.53 ± 0.05**C**	0.50 ± 0.05**bB**	0.44 ± 0.04**aA**
	Length	30	-	1.99 ± 0.44**aAB**	1.98 ± 0.56**aAB**	2.03 ± 0.43**aA**	1.73 ± 0.17**aB**
		35	-	1.97 ± 0.38**bA**	1.46 ± 0.25**bC**	1.55 ± 0.28**bB**	1.83 ± 0.20**abA**
		40	-	1.69 ± 0.23**cA**	1.34 ± 0.19**bB**	1.57 ± 0.21**bA**	1.91 ± 0.21**bC**

Different lowercase letters in a column indicate a significant difference among three LED height treatments according to Tukey's post-hoc test ($P < 0.05$).

Different uppercase letters in a row indicate a significant difference among four different nutrient concentration treatment according to Tukey's post-hoc test ($P < 0.05$). 0% nutrient concentration as negative control is excluded in statistical analyses.

30, 35 and 40 indicate LED height is 30cm, 35cm and 40cm from plants, respectively.

3. CONCLUSIONS

In this study, *M. crystallinum* achieved highest growth rates under 100% nutrient concentration, and 30cm LED height. 100% nutrient concentration is the ideal nutrient concentration for the growth of *M. crystallinum*. The 30cm LED height with the mean 222 PPFD is suggested as the optimum light for the early growing stage (40 days) of *M. crystallinum* in the industry. In plant pigment, 50% nutrient concentration achieved the highest chlorophyll a, chlorophyll b and carotenoid concentration in all LED height treatments. Instead of targeting the crop's yield, 50% nutrient concentration is suggested for the healthier production of *M. crystallinum.* This study also found that 75% nutrient concentration has the largest EBC size in all LED height treatments. The large EBCs size indicates the better juiciness and crunchiness of plant texture for the consumers. Further study of EBCs size in relation to sensory acceptance of *M. crystallinum* is required.

4. REFERENCES

Agarie, S., Shimoda, T., Shimizu, Y., Baumann, K., Sunagawa1, H., Kondo, A., Ueno, S., Nakahara, T., Nose1, A. & Cushman, C.J. (2007). Salt tolerance, salt accumulation, and ionic homeostasis in an epidermal bladder-cell-less mutant of the common ice plant *Mesembryanthemum crystallinum*. *Journal of Experimental Botany*. 58(8), 1957–1967.

Atzori, G., Vos, C.D.A., Rijsselberghe, V.M., Vignolini, P., Rozema, J., Mancuso, S. & Bodegom, M.V.P. (2017). Effects of increased seawater salinity irrigation on growth and quality of the edible halophyte *Mesembryanthemum crystallinum* L. underfield conditions. *Agricultural Water Management*. 187, 37–46.

Cha, M.K., Kim, J.S., Shin, J.H., Son, J.E., & Cho, Y.Y. (2014). Practical design of an artificial light-used plant factory for common ice plant (*Mesembryanthemum crystallinum* L.). *Protected Horticulture and Plant Factory*. 23(4), 371–375.

Cha, M.K., Park, K.S. & Cho, Y.Y. (2016). Growth characteristics of common ice plant. *Mesembryanthemum crystallinum* l. on nutrient solution, light intensity and planting distance in closed-type plant production system. *Protected Horticulture and Plant Factory*. 25 (2), 89–94.

Fevria, R., Vauzia, V., Farma, S.A.,Kardiman, R. & Edwin,E. (2023). The effect of eco-enzyme spraying on chlorophyll content of hydroponic lettuce (*Lactuca sativa* L.). *Proceedings of the 3rd International Conference on Biology, Science and Education* . 297–303.

Hammer, Ø., Harper, D. & Ryan, P. (2001). PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1):1–9.

He, J., You, X., & Qin, L. (2021). High salinity reduces plant growth and photosynthetic performance but enhances certain nutritional quality of C4 halophyte *Portulaca oleracea* l. Grown hydroponically under LED lighting. *Frontiers in Plant Science*. 12, 1–16.

He, J., Qin, L., Chong, E.L.C., Choong, T.W. & Lee, S.K. (2017). Plant growth and photosynthetic characteristics of *Mesembryanthemum crystallinum* grown aeroponically under different blue- and red-LEDs. *Frontiers in Plant Science*. 8. 361, 1–13.

Ibtissem, B., Abdelly, C. & Sfar, S. (2012). Antioxidant and antibacterial properties of *Mesembryanthemum crystallinum* and *Carpobrotus edulis* extracts. *Advances in Chemical Engineering and Science*. 2, 359–365.

刘士哲. (2001).*现代实用无土栽培技术*. 中国农业出版社.

Kim, Y.J., Kim, H.M., Kim, H.M., Jeong, B.R., Lee, H.J., Kim, H.J., & Hwang, S.J. (2018). Ice plant growth and phytochemical concentrations are affected by light quality and intensity of monochromatic light-emitting diodes. *Horticulture, Environment and Biotechnology*. 59, 529–536.

Moog, M.W., Trinh, M.D.L., Nørrevang, A.F., Bendtsen, A.K., Wang, C., Østerberg, J.T., Shabala, S., Hedrich, R., Wendt, T. & Palmgren, M. (2022). The epidermal bladder cell-free mutant of the salt-tolerant quinoa challenges our understanding of halophyte crop salinity tolerance. New Phytologist. 236(4), 1409-1421.

Young, J. K., Hye, M. K., Hyeon, M. K., Hye, R. L., Byoung, R. J., Hyeon-Jeong, L., Hyun-Jin, K., & Seung, J. H, (2021). Growth and phytochemicals of ice plant (*Mesembryanthemum crystallinum* L.) as affected by various combined ratios of red and blue LEDs in a closed-type plant production system. *Journal of Applied Research on Medicinal and Aromatic Plants*. 20, 1–8.

Thompson, E. (2022, August 15). *Mesembryanthemum crystallinum (crystalline iceplant)*. CABI Digital Library. https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.115578

United Nations Department of Economic and Social Affair. (2022, July 15). *World Population Prospects: Summary of Results.* https://www.un.org/development/desa/pd/.

Weeplian, T. & Ho, Y.S. (2014). Effects of photoperiod and photosynthetic photon flux on seed germination and growth of *Mesembryanthemum crystallinum* l. seedling. *International Conference on Plant Factory*.

Weeplian, T. & Yen, T-B., Ho, Y-S. (2018). Growth, development, and chemical constituents of edible ice plant (*Mesembryanthemum crystallinum* l.) Produced under combinations of light-emitting diode lights. *HortScience.* 53(6), 865–874.

Xia, J. & Mattson, N. (2022). Response of common ice plant (*Mesembryanthemum crystallinum* l.) To sodium chloride concentration in hydroponic nutrient solution. *HortScience.* 57(7), 750–756.

Woodward, R.B., Ayer, W.A., Beaton, J.M., Bickelhaupt, F., Bonnett, R., Buchschacher, P. & Volz, H. (1960). The total synthesis of chlorophyll. *Journal of the American Chemical Society*. 82(14), 3800–3802.