Implementation of ice plant (*Mesembryanthemum crystallinum* L.) production under semi-controlled conditions

Implementación de la producción de lechuga glacial (*Mesembryanthemum crystallinum* L.) bajo condiciones semicontroladas

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ABSTRACT

Ice plant (*Mesembryanthemum crystallinum* L.) is regarded as a drought and saline stress-tolerant plant with many biological properties, and especially valued in gourmet cuisine. The objective of this work was to find an optimum plant cultivation mode to produce edible parts under greenhouse conditions. Three soilless media were evaluated: peat, vermiculite and hydroponic culture. Peat pot culture yielded reduced biomass. Vermiculite and hydroponics, however, led optimum *M. crystallinum* growth. Plants grown in vermiculite and irrigated with nutrient solution presented a significantly higher yield. In fact, vermiculite-grown plants presented enhanced leaf area and leaf fresh weight as well as high foliar N, Mg, Mn, Fe, Na, chlorophyll and carotenoid concentrations. To conclude, greater succulence and Na concentration in edible parts of the vermiculite-grown glacier lettuce can offer consumers a more interesting taste, consistency and improved nutrient contents.

Keywords

Mesembryanthemum crystallinum • hydroponic culture • substrates • pot culture

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RESUMEN

La lechuga glacial (Mesembryanthemum crystallinum L.) muestra tolerancia a los estreses salino e hídrico, y posee diversas actividades biológicas que han permitido su revalorización como planta comestible en la alta cocina. El objetivo del presente trabajo fue evaluar el óptimo modo de cultivo para la producción de hojas de *M. crystallinum* bajo condiciones de invernadero. Para ello, se compararon tres formas de cultivo sin suelo: turba, vermiculita y cultivo hidropónico. El crecimiento en maceta con turba no resultó ser adecuado para la lechuga glacial, provocando una baja producción de biomasa. Sin embargo, tanto el cultivo en vermiculita como hidropónico permitieron un óptimo crecimiento de *M. crystallinum*, mostrando una producción nutritiva. La lechuga glacial desarrollada en vermiculita incrementó su área y materia fresca foliar, junto con elevadas concentraciones de N, Mg, Mn, Fe, Na, clorofilas y carotenoides en hojas. Además, el aumento de la suculencia y la concentración de Na en las partes comestibles de las plantas cultivadas en vermiculita pueden ofrecer un sabor, consistencia y contenido en nutrientes de mayor interés para el consumidor.

Palabras clave

Mesembryanthemum crystallinum • cultivo hidropónico • sustratos • cultivo en maceta

INTRODUCTION

Mesembryanthemum crystallinum, commonly known as ice plant or glacier lettuce, is regarded as a stress-tolerant plant. During the dry season and periods of water stress, the photosynthesis mode switches from C_3 to Crassulacean Acid Metabolism (CAM) (9). This halophytic plant possesses specialised trichomes called epidermal bladder cells, which have various functions including: water storage, salt accumulation, protection from UV rays and a role in plant defence (7). When mature plants die, the stored salt is leached into the soil thereby dramatically increasing salinity, making it difficult for other native species to grow (10, 20, 32). However, due to its ability to accumulate salt, the plant has been employed for soil desalination and proposed for bioremediation (2). The ice plant can also rapidly absorb soil moisture and help to build up high nitrate levels (13).

The ice plant's numerous biological properties include antioxidant activities, performed by betacyanin and other flavonoids, as well as antimicrobial activities (18, 25). Leaves and stems are used raw or cooked. With its succulent, mellow, slightly salty-tasting leaves, the species is considered a fashionable plant sold in delicatessen shops (16, 30). The product, however, is highly perishable. The wild nature of this species suggests it could be cultivated throughout the year, with little watering and care, based on a different maintenance approach (1). Therefore, the objective was to conduct a greenhouse production experiment and assess optimum ice plant growth conditions in soilless media.

MATERIALS AND METHODS

Plant material and growth conditions

Mesembryanthemum crystallinum seeds were collected from wild plants in Alicante (in South East Spain). They were surface disinfected with 0.5% NaClO for 2 h and pre-hydrated with aerated, distilled water for 22 h. Germination subsequently took place in vermiculite, hydrated with distilled water and maintained in a growth chamber at 24°C day/night (D/N) air temperature (T) and 70% D/N relative humidity (RH) (29). Chamber light conditions were 16 h light-8 h dark cycle with photosynthetically active radiation of 400 µmol m⁻²s⁻¹, provided by a combination of fluorescent tubes (Philips TLD 36W/83, Germany and Silvana F36W/GR0, USA). After 20 days, seedlings were transferred to a greenhouse under semi-controlled conditions of T D/N: 25/18°C; RH D/N: 60/80% and received natural daylight (mean photosynthetic photon flux rate of 400 µmol m⁻²s⁻¹) (3) (Photo 1, page 49). During the transplant, plants were divided into three homogeneous groups of 10 plants. Each group underwent different growth conditions: 1) seedlings transplanted to plastic

peat pots (V=1 L) watered weekly with 300 mL demineralised water; 2) seedlings inserted into 1 L plastic vermiculite pots watered weekly with 300 mL Hoagland nutrient solution (17); 3) seedlings grown in hydroponic containers (V=1 L) with Hoagland aerated nutrient solution, which was replaced weekly. Hoagland solution has been widely used for glacier lettuce growth (3, 5, 24, 29). Peat was mainly composed of pine bark, with 86% of organic matter based on dry weight, 49 mS m⁻¹ conductivity, pH 7 and grain size <10 mm. After 45 days, the plants were harvested and the different determinations were performed. At the end of the experiment, the electrical conductivity (EC) of the hydroponic, vermiculite and peat culture was 1.38, 0.57 and 0.63 mS cm⁻¹, respectively.



Photo 1. Experimental site and ice plants grown under different mode of cultivation.Foto 1. Unidad de experimentación vegetal y plantas de lechuga glacial crecidas bajo diferentes modos de cultivo.

Growth parameters

Plant dry weight (DW) was determined after oven drying at 80°C until constant weight. Shoot and root lengths were also measured. Leaf area was measured using the app "Easy Leaf Area Free" (12), while Specific leaf area (SLA) was calculated as the ratio of the leaf area to leaf dry weight.

Water status

Leaf relative water content (RWC) was calculated according to Weatherley's method (1950), using the following equation:

where: FW = fresh weight, TW = turgid weight, DW = dry weight of the tissue, respectively.

This determination was performed on expanded young leaves collected at noon. Foliar succulence was measured according to Atzori *et al.* (2017) as the ratio of leaf FW to leaf area.

Photosynthetic pigments

Foliar photosynthetic pigment concentration was determined in young, recently expanded leaves collected at noon, as described in Sesták *et al.* (1971). Samples (20 mg FW) were placed in 5 ml of 96% ethanol at 80° C for 10 minutes to extract the pigments. The absorbance of the extracts was spectrophotometrically measured and the equations reported by Lichtenthaler (1987) were used to calculate chlorophyll and carotenoid concentrations.

Mineral analysis

Leaf samples (0.5 g DW) were dry-ashed and dissolved in HCl in accordance with Duque (1971). Phosphorus, potassium, calcium, magnesium, manganese, zinc, iron and sodium concentrations were determined using a Perkin Elmer Optima 4300 inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer, USA). The ICP-OES operating parameters were: radio frequency power 1300 W, nebulizer flow 0.85 L min⁻¹, nebulizer pressure 206.84 kPa, auxiliary gas flow 0.2 L min⁻¹, sample introduction 1 mL min⁻¹ and three replicates per sample. Total nitrogen and carbon were quantified after leaf DW combustion (950°C) with pure oxygen using an elemental analyser with a thermal conductivity detector (TruSpec CN, Leco, USA).

Statistics

To compare all three treatments, a one-way analysis of variance (ANOVA) (SPSS v.26, IBM Corp., USA) was conducted. The means \pm standard deviation (SD) were calculated. When the *F*-ratio was significant (*p*<0.05), Duncan's multiple range test was applied. When only two treatments were compared, Student's *t*-test was performed. Significance levels were always set at 5%.

RESULTS

Growth parameters of *Mesembryanthemum crystallinum* showed significant differences according to the culture medium (table 1). Plants in vermiculite presented greater shoot growth and leaf DW than those under hydroponic conditions, but the difference was especially notable compared to peat-grown plants. Vermiculite treatment also enhanced ice plant root length and biomass, while peat growth presented significantly low values. In fact, peat pot culture did not seem to be an adequate substrate to cultivate *M. crystallinum*. Despite the same environmental conditions and age, plants did not grow properly. Therefore, it was not possible to obtain sufficient plant matter from the peat treatment to perform further determinations such as: RWC, succulence, photosynthetic pigments or mineral analysis. On the other hand, vermiculite-grown plants exhibited a significant increase in leaf area compared to the hydroponic culture, despite their similar SLA values.

Table 1. Growth parameters in ice plants grown under different mode of cultivation.

 Tabla 1. Parámetros de crecimiento de lechuga glacial crecida bajo diferentes modos de cultivo.

Treatment	¹ Shoot DW (g plant ⁻¹)	¹ Leaf DW (g plant ⁻¹)	¹ Root DW (g plant ⁻¹)	² Leaf area (cm ²)	¹ SLA (cm ² g ⁻¹)	² Shoot height (cm)	² Root length (cm)
Hydroponic	0.94±0.14 b	0.84±0.14 b	0.15±0.03 b	50,22±9,04 b	76,26±12,75 a	6.41±1.50 b	24.47±3.60 b
Peat	ND	ND	ND	0.87±0.14 c	ND	0.80±0.21 c	4.13±0.96 c
Vermiculite	2.12±0.36 a	1.83±0.29 a	0.32±0.07 a	133.65±19.14 a	72,47±16.94 a	10.25±2.04 a	29.36±5.16 a

¹Means (n=10) \pm SD were compared with Student-*t* test to analyze the differences between two of plant culture conditions. ²Means (n=10) \pm SD were compared with Duncan's test. Within each column, data followed by the same letter indicated that values did not differ significantly ($p \ge 0.05$). DW: dry weight; ND: not detected.

¹Para la comparación de dos condiciones de cultivo vegetal, las medias (n=10) ± DS fueron analizadas mediante el test *t*-Student. ² Las medias (n=10) ± DS fueron analizadas mediante el test de Duncan. Dentro de cada columna, el valor acompañado por una misma letra indica que las medias no difieren significativamente (p≥0,05). DW: materia seca; ND: no detectado.

Regarding leaf water status, ice plant foliar RWC did not differ according to the cultivation mode (table 2, page 51). Vermiculite-grown plants, however, presented the highest edible leaf FW production values together with greater succulence. As for photosynthetic pigments (table 3, page 51), both chlorophyll and carotenoid concentrations increased in vermiculite-grown plants.

Foliar mineral concentration is summarised in tables 4 and 5 (page 51). While C concentration did not differ among the plants grown in different media, foliar N concentration was higher in the ice plant cultivated in vermiculite (table 4, page 51). Moreover, vermiculite-grown plants had significantly higher Mg (table 4, page 51), Mn, Fe and Na (table 5, page 51) concentrations compared to the hydroponic treatment. Nevertheless, K and Zn concentrations were greater in plants grown in hydroponics, while P and Ca contents were similar across all treatments.

Table 2. Leaf fresh weight (FW), relative water content (RWC) and succulence in ice plants grown under different mode of cultivation.

Tabla 2. Materia fresca foliar, contenido hídrico relativo y suculencia de hojas de lechugaglacial crecida bajo diferentes modos de cultivo.

Treatment	Leaf FW (g)	RWC (%)	Succulence (g FW cm ⁻²)
Hydroponic	23.43±4.78 b	78.15±15.40 a	0.45±0.05 b
Vermiculite	66.53±16.19 a	88.18±7.48 a	0.51±0.04 a

Means (n=10) \pm SD were compared with Student-*t* test. Within each column, data followed by the same letter indicated that values did not differ significantly ($p \ge 0.05$).

Las medias $(n=10) \pm DS$ fueron analizadas mediante el test *t*-Student. Dentro de cada columna, el valor acompañado por una misma letra indica que las medias no difieren significativamente ($p \ge 0,05$).

 Table 3. Leaf photosynthetic pigment concentration in ice plants grown under different mode of cultivation.

 Tabla 3. Concentración foliar de pigmentos fotosintéticos de lechuga glacial crecida bajo diferentes modos de cultivo.

Treatment	Photosynthetic pigments (mg g ⁻¹ leaf DW)				
Treatment	Chlorophylls	Carotenoids			
Hydroponic	15.74±1,99 b	2.85±0.40 b			
Vermiculite	20.07±3.08 a	3.84±0.92 a			

Means (n=10) \pm SD were compared with Student-*t* test. Within each column, data followed by the same letter indicated that values did not differ significantly ($p \ge 0.05$). DW: dry weight.

Las medias (n=10) ± DS fueron analizadas mediante el test *t*-Student. Dentro de cada columna, el valor acompañado por una misma letra indica que las medias no difieren significativamente (*p*≥0,05). DW: materia seca.

Table 4. Foliar concentrations of macronutrients in ice plants grown under different mode of cultivation.

Tabla 4. Concentración foliar de macronutrientes de lechuga glacial crecida bajodiferentes modos de cultivo.

Treatment	C (mg g^{-1} DW)	N (mg g ⁻¹ DW)	P (mg g ⁻¹ DW)	K (mg g ⁻¹ DW)	Ca (mg g ⁻¹ DW)	Mg (mg g ⁻¹ DW)
Hydroponic	395.36±26.61 a	52.11±7.64 b	4.57±1.33 a	124.46±27.11 a	4.83±0.74 a	4.86±1.77 b
Vermiculite	373.91±35.75 a	64.18±3.79 a	4.20±0.46 a	75.05±13.50 b	4.84±0.85 a	13.26±1.32 a

 $\begin{array}{l} \mbox{Means (n=10) \pm SD were compared with Student-t test. Within each column, data followed by the same letter indicated that values did not differ significantly (p>0.05). DW: dry weight. \end{array}$

Las medias (n=10) ± DS fueron analizadas mediante el test *t*-Student. Dentro de cada columna, el valor acompañado por una misma letra indica que las medias no difieren significativamente (*p*≥0,05). DW: materia seca.

Table 5. Foliar concentrations of micronutrients and sodium in ice plants grown underdifferent mode of cultivation.

Tabla 5. Concentración foliar de micronutrientes de lechuga glacial crecida bajodiferentes modos de cultivo.

Treatment	Mn (μg g ⁻¹ DW)	Zn (μg g ⁻¹ DW)	Fe (μg g ⁻¹ DW)	Na (mg g ⁻¹ DW)
Hydroponic	84.43±15,93 b	129.34±25.84 a	128.66±44.36 b	5.26±1.47 b
Vermiculite	107.91±29.18 a	64.72±16.44 b	1509.37±339.51 a	31.97±8.97 a

 $\begin{array}{l} \mbox{Means (n=10) \pm SD were compared with Student-t test. Within each column, data followed by the same letter indicated that values did not differ significantly (p>0.05). DW: dry weight. \end{array}$

Las medias (n=10) ± DS fueron analizadas mediante el test *t*-Student. Dentro de cada columna, el valor acompañado por una misma letra indica que las medias no difieren significativamente (*p*≥0,05). DW: materia seca.

DISCUSSION

Peat is a widely used substrate for plant cultivation. In fact, *Mesembryanthemum crystallinum* has been successfully grown in peat-based substrate or mixed with vermiculite (3, 4, 8). However, our results showed that peat was not a suitable medium for ice plant pot-growing. Plant growth was very poor, making it impossible to determine many parameters and leading to significantly reduced shoot length, root length and leaf area. These results could be given by the fact that peat-grown plants were irrigated with demineralised water, while those grown in vermiculite and hydroponics received Hoagland nutrient solution. This may have generated an essential nutrient deficit in peat-grown plants, since the limited 1 L potting space increases nutrient needs (28). Moreover, according to Morales and Casanova (2015), peat is poorly drained. This probably leads to insufficient root oxygenation, causing growth limitations.

On the other hand, hydroponic and vermiculite cultures led to optimum *M. crystallinum* growth. In addition, vermiculite-grown plants irrigated with Hoagland nutrient solution presented a significantly greater yield. Vermiculite has a high cation exchange capacity (19), while in hydroponics, the nutrients are very accessible (22). It is worth noting that the hydroponic culture's EC levels at the end of the experiment were more than two times greater than that of the other two substrates; despite this, EC levels were within an optimum range regarding root water and nutrient absorption (15). In addition, the nutrient solution was replaced every week in order to ensure good nutrient balance in the hydroponic culture.

Vermiculite-grown plants presented higher values across all growth parameters, except for SLA. The reason may be that the nutrient intake of vermiculite-grown ice plants is greater due to longer roots and higher root DM, which would improve water and nutrient acquisition and/or use efficiency (26). In fact, plants grown in vermiculite presented higher leaf N, Mg, Mn and Fe concentrations.

Regarding the water status, cultivation mode was not found to influence foliar RWC in any way. On the contrary, leaf succulence improved in vermiculite-grown plants, probably due to greater nutrient intake than in hydroponics. Increases in succulence, that is, water content per unit area, allows osmotic adjustment (14). According to our results, vermiculite-grown *Mesembryanthemum* leaves also exhibited higher Na concentrations. The latter increases the salty taste of leaves and is highly appreciated by consumers. In fact, leaf succulence and glistening bladder cells, in particular, provide the edible leaves with a taste, consistency and appearance that make ice plants particularly valued by consumers (6). In addition, Agarie *et al.* (2007) worked on an ice plant mutant lacking epidermal bladder cells (EBCs). The authors concluded that EBCs contribute to succulence by serving as a water storage reservoir. They also contribute to salt tolerance by maintaining ion sequestration and homeostasis within photosynthetically active tissues of *M. crystallinum*.

Significant differences were found regarding pigment concentrations, with enhanced levels in total chlorophylls and carotenoids of vermiculite-grown leaves. This fact could imply that ice plants grown in vermiculite have a greater photosynthetic capacity, together with a greater allocation of energy in thermal dissipation over photochemistry (6).

CONCLUSIONS

Clear evidence was found that vermiculite pot culture irrigated with nutrient solution allows obtaining *M. crystallinum* yield for edible purposes. Under greenhouse conditions, this cultivation mode leads to greater biomass - including the production of edible fresh leaves - than hydroponics or peat culture. In addition, increased leaf succulence and Na concentration of vermiculite-grown plants offer consumers a more interesting taste, consistency and appearance.

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