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Agar concentration interferes with the biometry, photosynthetic pigment content, and anatomy of *Selenicereus undatus in vitro*

Concentração de ágar interfere na biometria, no conteúdo de pigmentos fotossintéticos e na anatomia de *Selenicereus undatus in vitro*

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ABSTRACT

The micropropagation of dragon fruit (*Selenicereus undatus*) is an alternative method to produce vigorous plants with high phytosanitary quality. However, depending on the consistency of the growing medium (liquid, semi-solid, and solid), plants can develop physiological and anatomical disorders, impairing their growth and reducing their viability *ex vitro*. The aim of this study was to evaluate the biometric characteristics, photosynthetic pigments, and anatomical sections of *S. undatus* plants grown in five concentrations of agar (0.0, 3.5, 7.0, 10.5, and 14.0 g L^{-1}) in MS medium. Biometric characteristics (number of roots, length of the aerial part, and fresh and dry masses of the roots and aerial part of the plants), photosynthetic pigments (chlorophyll *a*, *b*, total and carotenoid contents), and anatomy [number of vascular bundles, cross-sectional area (mm^2), and length of reserve parenchyma (µm)] were evaluated. Biometric, photosynthetic pigment, and anatomical characteristics differed between *S. undatus* plants for the different concentrations of agar. Plants grown in agar-free medium showed increased length, aerial and root biomass, indicating enhanced growth. In contrast, plants grown on media containing 10.5 g L^{-1} and 14.0 g L^{-1} agar exhibited higher levels of chlorophyll *a*, chlorophyll *b*, and total chlorophyll. Additionally, carotenoid levels were higher in plants grown on agar, regardless of concentration. Plants grown without agar showed higher vascular bundle count, cross-sectional area, and reserve parenchyma length than those with agar. In this way, *S. undatus* plants can be micropropagated efficiently in an agar-free medium.

Index terms: Cactaceae; dragon fruit; micropropagation; reserve parenchyma; vascular bundles.

RESUMO

A micropropagação de *Selenicereus undatus* (fruta do dragão) é uma alternativa para produzir plantas vigorosas com alta qualidade fitossanitária. No entanto, a consistência do meio de cultura (líquido, semissólido ou sólido) pode causar distúrbios fisiológicos e anatômicos, comprometendo o crescimento e a viabilidade *ex vitro*. O objetivo deste estudo foi avaliar as características biométricas, pigmentos fotossintéticos e anatomia de plantas de *S. undatus* cultivadas em meio MS com diferentes concentrações de ágar (0,0, 3,5, 7,0, 10,5 e 14,0 g L^{-1}). Foram analisados número de raízes, comprimento da parte aérea, massas frescas e secas, pigmentos fotossintéticos (clorofila *a*, *b*, total e carotenoides) e parâmetros anatômicos (número de feixes vasculares, área da seção transversal e comprimento do parênquima de reserva). Os resultados mostraram diferenças significativas entre as concentrações de ágar. Plantas cultivadas sem ágar apresentaram maior crescimento em comprimento, biomassa aérea e radicular. Já aquelas em meios com 10,5 e 14,0 g L^{-1} de ágar exibiram maiores níveis de clorofilas *a*, *b* e total. Independentemente da concentração, plantas cultivadas com ágar apresentaram maior conteúdo de carotenoides. Plantas sem ágar tiveram maior número de feixes vasculares, área da seção transversal e comprimento do parênquima de reserva. Conclui-se que *S. undatus* pode ser micropropagada de forma eficiente em meio líquido, sem a adição de ágar, otimizando o crescimento e as características anatômicas.

Termos para indexação: Cactaceae, fruta do dragão; micropropagação; parênquima de reserva; feixes vasculares.

Introduction

The use of plant biotechnology has made it possible to develop alternatives to multiplying plant species with a focus on their conservation, adaptation to climate change, and faster food production (Ollas et al., 2019; Munaweera et al., 2022). The biotechnological technique of *in vitro* cultivation, widely used for plant propagation, consists of cultivating plant cells or tissues inoculated in artificial nutrient media (liquid, semi-solid, or solid) under controlled environmental and axenic conditions for regeneration or vegetative multiplication (Ilyushko et al., 2020; Delgado-Paredes et al., 2021; Masoabi, Snyman, & Van der Vyver, 2023).

Under controlled conditions, the growing medium provides plants with mineral nutrients and other substances necessary for its growth and development and often for the regulation of cellular processes (Phillips & Garda, 2019; Souri & Hatamian,

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2019). However, certain components of the growing medium, such as the gelling agent agar, due to the quantity used, not only increase the costs of producing seedlings but can also hinder the growth and regeneration of vegetative material from different species. Depending on the fluidity of the medium (liquid, semi-solid, and solid), plant species can develop physiological and anatomical disorders in their tissues due to variations in nutrient uptake, oxygen availability, and mechanical support, which can disrupt normal growth and development (Aguilar et al., 2019; García-Ramírez, 2023; Manokari et al., 2023). For example, high water potential and/or high humidity in the medium can hinder the establishment of plants *in vitro* (Dubois & Inzé, 2020; Teixeira da Silva et al., 2020). A large amount of water in the growing medium can affect the growth rate, causing high accumulation of water in the apoplast, closure of stomata, irregular cell arrangement, and large intercellular spaces in plants (Kemat, Visser, & Krens, 2023).

The red dragon fruit (*Selenicereus undatus*), from the Cactaceae family, has high nutritional value (Yadav et al, 2024) and is widely cultivated in several countries (Betancur, Muriel, & González, 2020; Safira et al., 2021). The red dragon fruit (Hylocereus spp.) can be propagated through various methods, including both sexual (via seeds) and asexual means (Singh & Rani, 2023). Asexual propagation techniques include stem cuttings, grafting, and micropropagation, each offering unique advantages for efficient plant production (Borchetia, Neog, & Dutta, 2022).

The vegetative multiplication of *S. undatus* has been done through *in vitro* cultivation, where small segments of cladodes from matrices plants are disinfested and inoculated into aseptic medium for the production of clones (Mande, Kumbhare, & Surana, 2023). Among the elements contained in the aseptic medium is agar, which is an essential gelling agent in plant tissue culture, providing stability and support to explants during *in vitro* culture (Purohit, Habibi, & Purohit, 2011). Many studies have highlighted the usefulness of agar in various plant regeneration protocols (Mohamed et al., 2021; Rajam, 2024). For example, it facilitates the proliferation of species such as *Rubus fruticosus* L. and *Persea americana* Mill., ensuring consistent shoot multiplication and rooting under optimized conditions (Hiti-Bandaralage, Hayward, & Mitter 2017; Clapa et al., 2023).

However, the *S. undatus* species can be affected by the high water potential and/or high humidity of the growing medium (Wang et al., 2019), which impairs its multiplication and vegetative propagation (Kakade, Morade, & Kadam, 2022; Mori et al., 2023). Nevertheless, it is important to note that plant tissue culture allows for rapid multiplication, the production of disease-free plants, and the conservation of rare species, while also providing genetic uniformity and optimal control, making it a key tool in agriculture and biotechnology (Abdalla et al., 2022; Ozyigit et al., 2023).

The aim of this study was to evaluate the biometric characteristics, photosynthetic pigments, and anatomical sections of *S. undatus* plants grown *in vitro* in five concentrations of agar (0.0, 3.5, 7.0, 10.5, and 14.0 g L^{-1}) in MS medium.

Material and Methods

The experiment was conducted at the Plant Tissue Culture Laboratory in the Agriculture Department of the Federal University of Lavras (UFLA) in Lavras, state of Minas Gerais, Brazil.

Plant material and *in vitro* cultivation

Cladodes of dragon fruit (*Selenicereus undatus*), about 1.5 cm long, were used as explants and grown in 250 mL flasks containing 50 mL of MS medium (Murashige & Skoog, 1962) plus 30 g L^{-1} sucrose and five concentrations of agar (0.0, 3.5, 7.0, 10.5, and 14.0 g L^{-1}) (Agargel Ind. e Comércio Ltda). The pH of the media was adjusted to 6.0 ± 0.2 and autoclaved at 121 °C and 1.2 atm pressure for 20 min. The five concentrations of agar resulted in five media, one of which was liquid (0%), one semi-solid (50%), and three solids (100%, 150%, and 200%).

Five explants per flask were inoculated in a laminar flow chamber, with 20 replicates per treatment. These flasks were kept for 56 days in a growth room with artificial lighting provided by white LED lamps and an average irradiance of 52.5 µmol m^{-2} s^{-1}, with a 16-hour photoperiod and a temperature of 25 ± 2 °C.

Biometric characteristics

The biometric characteristics were assessed on five plants and five flasks collected at random from each treatment after 56 days of *in vitro* cultivation. The number of roots, the length of the aerial part (cm), and the fresh and dry masses of the roots and aerial part (g $plant^{-1}$) of plants were evaluated.

The length was measured using a ruler graduated in millimeters, and the fresh and dry masses were quantified using a precision scale with four decimal places (OHAUS Model PR224BR). After measuring the fresh mass of the roots and aerial part, each was placed in individual paper bags and transferred to an oven (TECNAL Model TE-394/3 MP) with forced air circulation at 65 °C for 72 hours. The dry mass was quantified after removing the material from the oven and stabilizing the temperature in a Styrofoam box.

Photosynthetic pigments

Photosynthetic pigment analysis was carried out on fresh cladodes (± 0.050 g) from five *S. undatus* plants randomly collected from each treatment after 56 days of *in vitro* cultivation. The samples were transferred to test tubes containing 5 mL of 80% acetone for the extraction of chlorophylls and carotenoids. The test tubes were wrapped in aluminum foil to prevent the sample from coming into contact with light and to prevent chlorophyll degradation.

After 24 hours in a refrigerator at ± 4 °C, the samples were centrifuged for 5 min and measured for absorbance using an Elisa Multiskan GO spectrophotometer (Thermo Fisher Scientific)

at wavelengths of 470 (Abs470), 647 (Abs647), and 663 nm (Abs663) (Scopel, Barbosa, & Vieira, 2011). The chlorophyll *a*, *b*, total, and carotenoid contents were determined using the equations (Lichtenthaler & Wellburn, 1983; Chazaux et al., 2022). The wavelength readings were taken in five replicates per treatment, with each replicate being evaluated in triplicate. The analysis was carried out using Skanit Software 5.0 for Microplate Readers RE version 5.0.0.42.

Anatomical aspects

The anatomical aspects were assessed on fresh cladodes after eight weeks of *in vitro* cultivation, randomly collected from five plants of each treatment. The cladodes were fixed in FAA solution (formaldehyde, acetic acid, and 50% ethanol in a ratio of 0.05:0.05:0.90) for 72 hours. Initially, the plant material was gradually bleached with 70, 80, 90, and 100% ethyl alcohol (ethanol) every 2 hours (Johansen, 1940). The cladodes were placed for 72 hours in a solution of 50% alcohol and 50% pure resin (Historesin Leica) and fixed with the same type of material. The cladodes were embedded in a solution containing 3 mL of 15% pure resin, with 1% hardener added to facilitate cutting. Transverse and longitudinal sections, 9 µm thick, were made on a MRS 3500 semi-automatic rotary microtome in five replicates per plant.

The sections were stained with Toluidine blue solution (0.05%) and Sudan IV to identify lipid substances such as essential oil (Gerlach, 1984) and then mounted as permanent slides in glycerin gelatine (colorless gelatine + glycerin water).

The anatomical analyses carried out were counting the number of vascular bundles, determining the cross-sectional area (mm^2) and the length of reserve parenchyma (µm). The sections were observed under a light microscope (Nikon, Eclipse E100) coupled with a digital camera (Infinity, Bullet Full HD) to capture the images. Photomicrographs were used to measure anatomical features using UTHSCSA-Imagetool® software calibrated with a microscope.

Statistical analysis

The experiment was carried out in a completely randomized design. The data obtained was submitted to analysis of variance, and the means were compared using the Scott-Knott test at 5% probability using the SISVAR statistical analysis program (Ferreira, 2011).

Results and Discussion

The biometric characteristics assessed varied among *Selenicereus undatus* plants grown under different agar concentrations. The number of roots per plant was higher in the treatments with 3.5 and 7.0 g L^{-1} agar and lower in the other treatments (Table 1).

Table 1: Effect of agar concentration in MS medium on root number (NR) and aerial part length (APL) of *S. undatus* plants after 56 days of *in vitro* cultivation.

Agar (g L^{-1})	NR	LAP (cm)
0.0	5.35 ± 0.52b	4.56 ± 0.55a
3.5	5.80 ± 0.50a	2.98 ± 0.30b
7.0	6.55 ± 0.45a	2.57 ± 0.28b
10.5	4.40 ± 0.46b	2.80 ± 0.24b
14.0	5.10 ± 0.45b	2.80 ± 0.24b
C.V. (%)	19.08	15.47

Averages (± standard error). Mean values followed by the same letter in the row do not differ by the Scott-Knott grouping test at 5% probability. C.V. - coefficient of variation (%).

Other researchers (Torres-Silva et al., 2020; Matos et al., 2020) have also observed a higher number of roots in plants cultivated in medium containing 3.5 to 7.0 g L^{-1} of agar. Low concentrations of agar can reduce the number of roots emitted by plants (Chaves & Cunha, 2021), due to a lower amount of oxygen in liquid medium, which can impair cell proliferation and root growth (Shukla et al., 2020). The higher concentration of O_2 in media containing 3.5 to 7.0 g L^{-1} agar can favor root emission; however, environments with higher concentrations of agar can impair emission due to mechanical restrictions and lower nutrient availability (Assis et al., 2018; Leite et al., 2021; Dewir et al., 2023).

The length of the aerial part of the plants grown in agar-free medium was greater than in the other treatments (Table 1).

The greater length of the aerial part of the cladodes of *S. undatus* plants grown in MS medium without agar may be due to the greater availability of nutrients for absorption by the plants since minerals are dissociated in liquid medium (Dewir et al., 2023). On the other hand, higher levels of agar stiffen the medium, decreasing the water potential, preventing hyperhydration (hyperhydricity), limiting nutrient absorption, and, consequently, plant length (Dewir et al., 2023; Marhr et al., 2023).

The fresh mass of the roots of *S. undatus* plants varied between the concentrations of agar in the MS medium, with those grown with 7.0, 10.5, and 14.0 g L^{-1} having the highest averages (Table 2).

The greater fresh mass roots of *S. undatus* plants grown *in vitro* in media with higher concentrations of agar may be due to the cacti's greater adaptation to cultivation with lower water availability. *In vitro* cacti regulate their growth to absorb, accumulate, and transport water, improving their efficiency of use in conditions of greater water restriction (Radi et al., 2023). The lower fresh weight of the aerial part of plants grown with higher concentrations of agar may be related to the effects of water stress on plant growth *in vitro* or the lower bioavailability of minerals and growth factors (Souri & Hatamian, 2019; Lahijanian et al., 2023).

In addition, researchers have observed that concentrations of 10.5 and 14.0 g L^{-1} of agar in the culture medium reduce water availability within the vessels and cause lower plant growth by reducing the flow of nutrients to the meristematic points. However, low concentrations of agar in the medium enabled better osmotic adjustment in plants with nutrient accumulation and more balanced growth (Silva e Souza et al., 2023).

The fresh and dry mass of the aerial part was higher in the *S. undatus* plants from the treatment without agar (Table 2). The higher fresh and dry mass of the aerial part of the plants grown without the presence of agar in MS medium may be due to the higher free energy of the nutrient solution. The presence of a gelling agent in the medium reduces the free potential of water, making it more difficult for plants to absorb it (Mayahi & Ali, 2021). The lower the free energy of the water, the less it is absorbed by plants, which reduces or delays the absorption of nutrients and, consequently, impairs the accumulation of mass by plants (Manokari et al., 2023; Suthar, Habibi, & Purohit 2011; Das et al., 2015).

The dry mass roots of *S. undatus* plants was higher in those grown in liquid medium (0.0 g L^{-1} agar) (Table 2). The increased dry mass of roots in plants grown in MS medium without agar is likely attributed to low iron stress caused by reduced oxygen availability (Souri & Hatamian, 2019; Souri, Neumann, & Römheld, 2009). Studies on *Ocimum basilicum* shoots grown in B5 medium (Gamborg, Miller, & Ojima 1968) without agar had similar results (Monfort et al., 2018). Species from the Cactaceae family can more easily accumulate water and minerals from the growing medium, as they have cells with a more elastic cell wall (Fradera-Soler et al., 2022). This greater accumulation can result in an increase in the production of photoassimilates, which can lead to higher dry mass production (Bello-Bello et al., 2021).

The chlorophyll *a*, *b*, and total contents differed between *S. undatus* plants. The highest values were observed in plants grown in media with 10.5 and 14.0 g L^{-1} agar (Table 3).

The increase in photosynthetic pigment content in plants grown on solid media may be related to a lower accumulation of mass in the aerial part, causing a higher concentration of pigments per cladode unit area (Hajnal-Jafari et al., 2020). In addition, overhydrated plants can accumulate excess water in the cells or tissues with poor lignification of the bark, which can lead to cell enlargement with reduction in performance and photosynthetic pigments resulting in a transparent and glassy appearance and reduced mechanical strength (Borland et al., 2018; Souza et al., 2019; Bouzroud et al., 2022).

Table 2: Fresh and dry mass of roots (FMR, DMR) and aerial parts (FMAP, DMAP) in g $plant^{-1}$ of *S. undatus* at 56 days of *in vitro* cultivation under different agar concentrations in MS medium.

Agar (g L^{-1})	FMR	FMAP	DMR	DMAP
0.0	0.35 ± 0.05b	4.50 ± 0.59a	0.07 ± 0.01a	0.38 ± 0.04a
3.5	0.42 ± 0.04b	1.35 ± 0.11b	0.06 ± 0.01a	0.08 ± 0.01b
7.0	0.68 ± 0.04a	1.47 ± 0.15b	0.03 ± 0.01b	0.09 ± 0.01b
10.5	0.65 ± 0.04a	1.11 ± 0.13b	0.03 ± 0.01b	0.06 ± 0.01b
14.0	0.74 ± 0.02a	1.53 ± 0.09b	0.03 ± 0.01b	0.08 ± 0.01b
C.V. (%)	15.83	12.22	21.73	13.32

Averages (± standard error). Mean values followed by the same letter in the row do not differ by the Scott-Knott grouping test at 5% probability. C.V. - coefficient of variation (%).

Table 3: Chlorophyll *a* (Chla), *b* (Chlb), total (Chlt), and carotenoid (Car) content (µg g^{-1} fresh mass) in *S. undatus* plants at 56 days of *in vitro* cultivation under different agar concentrations in MS medium.

Agar (g L^{-1})	Chla	Chlb	Chlt	Car
0.0	0.42 ± 0.04b	0.10 ± 0.04b	0.53 ± 0.03b	0.11 ± 0.01b
3.5	0.98 ± 0.02b	0.13 ± 0.03b	1.11 ± 0.03b	0.35 ± 0.04a
7.0	1.36 ± 0.32b	1.19 ± 0.61b	2.55 ± 0.72b	0.31 ± 0.11a
10.5	5.58 ± 0.45a	4.55 ± 0.47a	10.13 ± 0.91a	0.40 ± 0.01a
14.0	5.81 ± 0.10a	4.70 ± 0.41a	10.50 ± 0.48a	0.35 ± 0.02a
C.V. (%)	17.85	16.65	22.70	16.06

Averages (± standard error). Mean values followed by the same letter in the row do not differ by the Scott-Knott grouping test at 5% probability. C.V. - coefficient of variation (%).

The carotenoid content was higher in the *S. undatus* plants grown in medium containing agar, without differing from each other, but from the plants without agar (Table 3). The higher carotenoid content observed in *S. undatus* plants grown in medium containing agar may be related to the occurrence of stress due to the presence of this gelling agent in the growing medium. Agar causes a reduction in the water potential of the medium, which leads to a reduction in the absorption of water and nutrients (Schorr, Ikeda, & de Alcantara, 2023). The higher concentration of carotenoids may be a defense mechanism for *S. undatus* plants under conditions of limited water in the tissues. This pigment helps to reduce the photooxidation of other photosynthetic pigments and the degradation of chlorophyll (Đurić et al., 2023), meaning that the plants synthesize more carotenoids that will act in the photoprotection of photosystems I and II (Cristina et al., 2023).

The anatomical characteristics of the plants varied between treatments (Figure 1 and Table 4).

The number of vascular bundles and the cross-sectional area were higher in the *S. undatus* plants grown without agar. The length of the reserve parenchyma had the highest average values in the plants without agar but did not differ from those in the 3.5 g L^{-1} concentration of agar in the medium (Table 4 and Figure 2).

The length of the reserve parenchyma of the cladodes of *S. undatus* plants was greater in plants grown with 0.0 and 3.5 g L^{-1} agar (Table 4 and Figure 2). The growth of cortex cells (reserve parenchyma) in cacti is related to their ability to expand and store water. When free water is available, these cells expand, occupying 50 to 70% of the cortex volume (Torres-Silva et al., 2020). Thus, the absence of agar or its low concentration leaves the medium with a higher water potential, being absorbed more easily by the plant tissues and accumulating in the parenchyma cells, favoring their expansion (Carlquist, 2018).

Cacti that grow in liquid environments may have a greater number of vascular bundles, cross-sectional area of cladodes, and proportion of parenchyma (Montathong, Machikowa, & Muangsan 2019; Ma et al., 2023); these structures, in greater number and size, may be a consequence of the accumulation of water in the tissues of the plants (Torres-Silva et al., 2020). Some species have developed adaptation and mitigation strategies against water stress due to excess humidity, improving their aqueous solution storage capacities in specific structures such as reserve parenchyma, vascular bundles, and aerenchyma (Fang & Xiong, 2015; Khan et al., 2020; Mignolli, Todaro, & Vidoz, 2020). In addition, it has been shown that in anaerobic conditions, with excess water, plants are stimulated to synthesize higher concentrations of the plant hormone ethylene. This hormone causes plants to produce more vascular bundles to increase aeration of the aerial part, in addition to being able to transport and retain water and nutrients to reduce excess water and its damage to plant tissues (Yang et al., 2021).

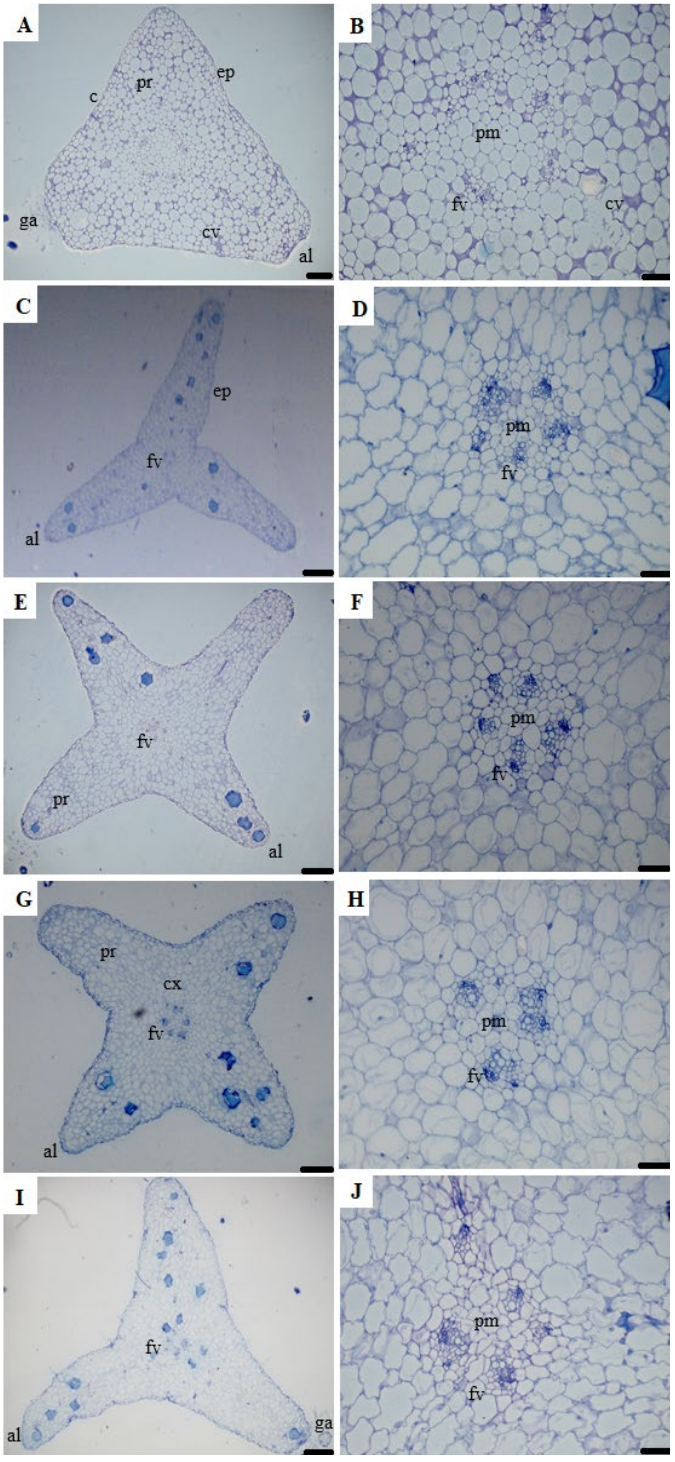

Figure 1: Cladode cross-sectional areas of *S. undatus* at 56 days of *in vitro* cultivation under different agar concentrations in MS medium [0.0 (A, B); 3.5 (C, D); 7.0 (E, F); 10.5 (G, H); 14.0 (I, J) g L^{-1}]. Legend: c = cuticle; ep = epidermis; pr = reserve parenchyma; pm = medullary parenchyma; cv = mucilaginous cavity; cx = cortex; al = ala; ga = axillary bud (areola); fv = vascular bundle. Scale bar = 100 µm.

Table 4: Vascular bundle number (NVB), cladode cross-sectional area (CSA), and reserve parenchyma length (LRP) of *S. undatus* at 56 days of *in vitro* cultivation under different agar concentrations in MS medium.

Agar (g L^{-1})	NVB	CSA (mm^2)	LRP (µm)
0.0	11.00 ± 0.40a	0.79 ± 0.01a	64.25 ± 1.40a
3.5	5.40 ± 0.10b	0.71 ± 0.01b	63.58 ± 1.30a
7.0	5.80 ± 0.20b	0.74 ± 0.01b	56.32 ± 0.80b
10.5	5.50 ± 0.20b	0.72 ± 0.01b	52.23 ± 1.30b
14.0	5.00 ± 0.01b	0.65 ± 0.01c	55.36 ± 1.20b
C.V. (%)	6.84	5.06	17.77

Averages (± standard error). Mean values followed by the same letter in the row do not differ by the Scott-Knott grouping test at 5% probability. C.V. - coefficient of variation (%).

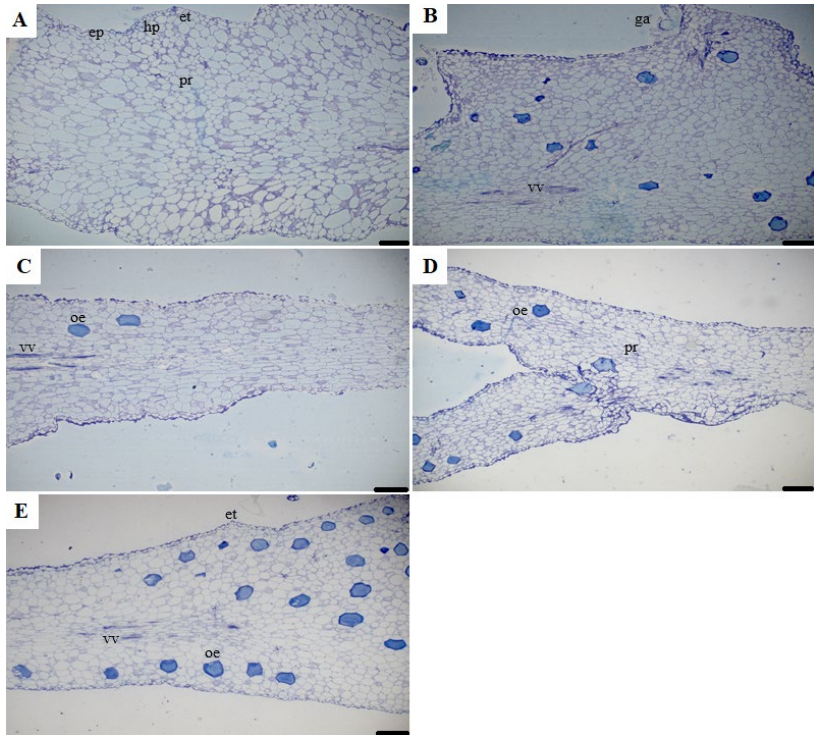

Figure 2: Longitudinal sections of cladodes of *S. undatus* at 56 days of *in vitro* cultivation under different agar concentrations in MS medium [0.0 (A); 3.5 (B); 7.0 (C); 10.5 (D); 14.0 (E) g L^{-1}]. Legend: (A) ep = epidermis; pr = reserve parenchyma; hp = hypodermis; et = stomata; (B) ga = axillary bud; vv = vascular vessel; (C) oe = essential oil; vv = vascular vessel; (D) oe = essential oil; pr = reserve parenchyma; (E) et = stomata; vv = vascular vessel; oe = essential oil. Scale bar = 100 µm.

Conclusions

The different concentrations of agar in the growing medium influenced the biometric characteristics, photosynthetic pigments, and anatomical aspects of *Selenicereus undatus* plants. Plants cultivated without agar demonstrated superior biometric and anatomical characteristics, while also lowering costs by utilizing a more economical growth medium.

Author Contributions

Conceptual idea: Evens, C.; Bruno, H.F.; Marcelo, A. G.; Methodology design: Evens, C.; Bruno, H.F.; Marcelo, A. G; Filipe, A. R.; Data collection: Evens, C.; Marcelo, A. G.; Data analysis and interpretation: Evens, C.; Marcelo, A. G.; Filipe, A. R.; Gabrielen, M. G. D., and Writing and editing: Evens, C.; Marcelo, A. G.; Filipe, A. R.; Joyce, D.; Gabrielen, M. G. D.; Evaristo, M. C.; Moacir, P.

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