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ORIGINAL ARTICLE

In vitro propagation and rooting strategies for *Hylocereus undatus* and *Hylocereus polyrhizus*

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Abstract

In vitro micropropagation protocols for *Hylocereus undatus* and *Hylocereus polyrhizus* pitaya species were developed in Murashige and Skoog (MS) and Lloyd and McCown (WPM) nutrient media. During micropropagation stage, 0, 3, 4, 5, 6 mgL^{-1} Benzyl adenine (BA), 0.2 mgL^{-1} Gibberellic acid (GA_3), and 0.1 mgL^{-1} Indole butyric acid (IBA) were added to the MS and WPM media. For the rooting stage, 0, 0.5, 1, 1.5, 2 mgL^{-1} IBA, 0.5 mgL^{-1} Naphthalene acetic acid (NAA), 0.2 mgL^{-1} GA_3 and 0.1 mgL^{-1} BA were added to the same media. *H. undatus* reached the highest number of shoots (8.94) in the MS medium containing 3 mgL^{-1} BA while *H. polyrhizus* achieved the highest number of shoots (4.46) in the MS medium containing 5 mgL^{-1} BA. The WPM medium (3.08) produced a higher average number of roots compared to the MS medium (2.55). The highest average root number was obtained from 0.5 mgL^{-1} IBA concentration, followed by 2 mgL^{-1} IBA concentration. *H. polyrhizus* (51.63 mm) had a higher average root length than *H. undatus* (49.59 mm). The MS medium (51.22 mm) was more effective in promoting average root length compared to the WPM medium (49.99 mm). The highest average shoot fresh weight (1183.33 mg) and shoot dry weight (106.00 mg) were obtained from *H. undatus* in the WPM medium containing 2.0 mgL^{-1} IBA. *H. polyrhizus* (399.97 mg) had a higher average root fresh weight than *H. undatus* (275.60 mg) with the 2 mgL^{-1} of IBA concentration yielding the highest average value (604.83 mg). *H. polyrhizus* (35.93 mg), the WPM medium (38.30 mg), and 2 mgL^{-1} of IBA concentration (49.75 mg) resulted in a higher average root dry weight.

Keywords Dragon fruit · Plant growth regulators · Multiplication · Tissue culture

Introduction

Pitaya species (*Hylocereus* spp.) belong to the Cactaceae family. Although their origin is Mexico and Central America, today, they are now mainly grown in Southeast Asian countries (Vietnam, Thailand), Israel, Australia, Nicaragua, and the USA. These species are widely distributed, from coastal areas to high mountains and tropical rainforests. Fruit peel color varies from red, pink, and yellow; fruit flesh color ranges from white to purple (Seday and Sanal 2017). The plants vary significantly in size, from ring-sized species to massive columnar types and epiphytic (climbing) species. Epiphytic pitaya plants use aerial roots on their trunks to cling to rocks and trees for support. Cacti are highly adaptable and can withstand drought, heat, and nutrient-poor soil conditions. In their native regions, these plants have been used for many purposes, but the most important use is as a food source, specifically the fruit. *Hylocereus undatus* is a climbing species thought to originate from the tropical rainforests of Central and Northern South America. *H. undatus* has a pink skin and white fruit flesh. The species *Hylocereus polyrhizus* also consists of climbing plants. *H. polyrhizus* has a red shell and flesh. Both species produce large, fragrant, night-blooming flowers. While *H. undatus* is a long-day plant, *H. polyrhizus* is not as dependent on day length and produces fruit most of the year (Luders and McMahon 2006).

Plant tissue culture involves producing new tissues, plants, or plant products under controlled environmental conditions using explants such as cells, tissues, or organs in an artificial nutrient medium under aseptic conditions (Babaoğlu et al. 2001). Micropropagation refers to the production of new plants from explants taken from a plant (embryo, seed, stem, shoot, root, callus, etc.) that have the potential to form a complete plant in artificial nutrient media

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and under aseptic conditions (Hartman and Kester 1975). Bürün and Türkoğlu (1996) reported that environmental conditions such as temperature, light, and photoperiod significantly influence the growth and development of plant materials in tissue culture studies. However, in many tissue culture studies, little importance has been given to determining the optimal cultural conditions.

The micropropagation process of pitaya includes the establishment of an aseptic culture from shoot tips and lateral buds, followed by shoot elongation, proliferation, and rooting. Pitaya can be propagated in vitro through both direct and indirect regeneration methods (Shah et al. 2023).

The first tissue culture study on pitaya was conducted by Infante (1992) on yellow pitaya (*Mediocactus coccineus*). Since then, many researchers have carried out studies on propagation and rooting with different species and varieties (Mohamed-Yasseen 2002; Drew and Azimi 2002; Dahanayake and Ranawake 2011; Vinas et al. 2012; Hua et al. 2015; Mukarlina 2015; Qin et al. 2017; Bozkurt et al. 2020; Delgado-Paredes et al. 2021; Usta 2022; Dewir et al. 2023), seed germination (Kari et al. 2010; Sheng et al. 2016), indirect organogenesis (Nunez et al. 2016; Lopes et al. 2016; Rodrigues et al. 2022), somatic embryogenesis (Roman et al. 2014) and regeneration (Pelah et al. 2002; Fan et al. 2013).

Micropropagation offers several advantages for propagating *Hylocereus* species, such as rapid, year-round production regardless of seasons and disease-free seedlings. However, successful application requires sterile laboratory conditions, qualified personnel, and specialized equipment. Therefore, it is important to experimentally optimize media compositions and develop appropriate protocols for the optimal growth and development of explants in vitro.

This research aims to develop in vitro propagation and rooting protocols for *H. undatus* and *H. polyrhizus* pitaya species. Thus, it is also aimed to provide convenience to producers in supplying fast and large quantities of saplings for pitaya cultivation, which has become widespread in the Mediterranean Region of Türkiye.

Materials and methods

Experimental design and plant growth conditions

The research was conducted at the Saadet Büyükalaca Tissue Culture Laboratory of Department of Horticulture, Faculty of Agriculture, Çukurova University in Adana, Turkiye. A completely randomized experimental design was employed for the propagation experiment with three factors (genotype (2 levels) x nutrient media (2 levels) x BA (5 levels)). The rooting experiment was also conducted using a completely randomized experimental design with three factors (genotype (2 levels) x nutrient media (2 levels) x IBA (5 levels)). Propagation and rooting experiments were set up with three replicates for each genotype with 9 explants in each replicate. A total of 20 different media were tested, 10 at the propagation stage and 10 at the rooting stage per each genotype. All cultures were maintained under 16/8 h (light/dark) photoperiod and at a temperature of 25 ± 2 ℃.

Plant material

The plant materials utilized in this study were areoles from two species: *H. undatus* and *H. polyrhizus*. The notable characteristics of these genotypes are as follows: *H.undatus* is a perennial species with a green, fleshy trunk. It exhibits nocturnal blooming, producing flowers that open only at night. The fruit of this species has a red skin, while the flesh is white. Additionally, it is self-productive and does not require cross-pollination for fruit development. *H polyrhizus*, a perennial species, also has a green, fleshy trunk and blooms exclusively at night. However, the fruit of *H. polyrhizus* is distinct in that both the skin and the flesh are red. This species is also self-productive, capable of fruiting without the need for cross-pollination.

Surface sterilization

The shoots were washed under running water for 20 min and then soaked in a 1 gL^{-1} fungicide containing benomyl as the active ingredient for 5 min. After being transferred to the sterile cabinet, the shoots were divided into smaller pieces, shaken in 70% ethyl alcohol for 1.5 min, and washed 3 times with sterile distilled water. Next, 1–2 drops of Tween 20 were added to a 15% sodium hypochlorite solution, and the shoots were shaken in this solution for 8 min. The areoles were then taken from the explants, washed 4–5 times with sterile distilled water, and the study was initiated. During the shoot proliferation stage, a medium consisting of ½ MS + ½ WPM + 1 mgL^{-1} BA + 0.2 mgL^{-1} GA_3 was used. Explants were subcultured every 5 weeks.

Propagation stage

During the propagation experiment, two different media, Murashige and Skoog (1962) (MS) and Lloyd and McCown (1980) (WPM), were used for both genotype. Each media had different concentrations of BA (0, 3, 4, 5, 6 mgL^{-1}), 0.2 mgL^{-1} GA_3, and 0.1 mgL^{-1} IBA. The compositions of the plant growth regulators (PGRs) of the nutrient medium used are given in Table 1. A total of 30 gL^{-1} sugar was used in the nutrient media. The pH of the media was adjusted to 5.8, and 7 gL^{-1} agar was added. The prepared media were heated on a heater until boiling started. The mixture was then distributed into glass jars (75 ml/jar) and sterilized in an autoclave at 121 °C and 1.05 kg cm^{-2} pressure for 15 min. At the end

Table 1 Nutrient medium and growth regulator combinations used at different stages in the experiment

	In the micropropagation stage		In the rooting stage	
1	MS	Without PGRs (control)	MS	Without PGRs (control)
2	WPM	Without PGRs (control)	WPM	Without PGRs (control)
3	MS	3 mgL^{-1} BA + 0.2 mgL^{-1} GA_3 + 0.1 mgL^{-1} IBA	MS	0.5 mgL^{-1} IBA + 0.5 L^{-1} NAA + 0.2 mgL^{-1} GA_3 + 0.1 mgL^{-1} BA
4	WPM	3 mgL^{-1} BA + 0.2 mgL^{-1} GA_3 + 0.1 mgL^{-1} IBA	WPM	0.5 mgL^{-1} IBA + 0.5 mgL^{-1} NAA + 0.2 mgL^{-1} GA_3 + 0.1 mgL^{-1} BA
5	MS	4 mgL^{-1} BA + 0.2 mgL^{-1} GA_3 + 0.1mgL^{-1} IBA	MS	1mgL^{-1} IBA + 0.5 mgL^{-1} NAA + 0.2 mgL^{-1} GA_3 + 0.1 mgL^{-1} BA
6	WPM	4 mgL^{-1} BA + 0.2 mgL^{-1} GA_3 + 0.1 mgL^{-1} IBA	WPM	1 mgL^{-1} IBA + 0.5 mgL^{-1} NAA + 0.2 mgL^{-1} GA_3 + 0.1 mgL^{-1} BA
7	MS	5 mgL^{-1} BA + 0.2 mgL^{-1} GA_3 + 0.1 mgL^{-1} IBA	MS	1.5 mgL^{-1} IBA + 0.5 mgL^{-1} NAA + 0.2 mgL^{-1} GA_3 + 0.1 mgL^{-1} BA
8	WPM	5 mgL^{-1} BA + 0.2 mg L^{-1} GA_3 + 0.1 mgL^{-1} IBA	WPM	1.5 mgL^{-1} IBA + 0.5 mgL^{-1} NAA + 0.2 mgL^{-1} GA_3 + 0.1 mgL^{-1} BA
9	MS	6 mgL^{-1} BA + 0.2 mgL^{-1} GA_3 + 0.1 mgL^{-1} IBA	MS	2 mgL^{-1} IBA + 0.5 mgL^{-1} NAA + 0.2 mgL^{-1} GA_3 + 0.1 mgL^{-1} BA
10	WPM	6 mgL^{-1} BA + 0.2 mgL^{-1} GA_3 + 0.1 mgL^{-1} IBA	WPM	2 mgL^{-1} IBA + 0.5 mgL^{-1} NAA + 0.2 mgL^{-1} GA_3 + 0.1 mgL^{-1} BA

of experiment, the number of shoots and shoot lengths (mm) were recorded in each 3 subculture.

Root induction

During rooting stage also, two different media, Murashige and Skoog (1962) (MS) and Lloyd and McCown (1980) (WPM), were used for both genotype. Each media had different concentrations of IBA (0, 0.5, 1, 1.5, 2 mgL^{-1}), 0.5 mgL^{-1} NAA, 0.2 mgL^{-1} GA_3 and and 0.1 mgL^{-1} BA. The compositions of the PGRs of the nutrient media used are given in Table 1. A total of 30 gL^{-1} sugar was used in the nutrient media. The pH of the media was adjusted to 5.8, before adding agar (7 gL^{-1}). The prepared media were heated on a heater until boiling started. The mixture was then distributed into glass jars (75 ml/jar) and sterilized in an autoclave at 121 °C and 1.05 kg cm^{-2} pressure for 15 min. After the rooting stage, the plantlets were removed from the jar, the roots were washed, and after removing moisture with filter paper, the shoots and roots were cut and separated. After recording the number of roots, root length (mm), root fresh weight (mg), and shoot fresh weight (mg), the materials were placed in an oven with compressed air circulation at 50 °C. Shoots and roots were dried for 120 h until a constant weight was achieved, and shoot dry weight and root dry weight were determined (Rodrigues et al. 2021).

Murashige and Skoog (1962) (MS) and Lloyd and McCown (1980) (WPM) media were used in the experiment.. In the propagation stages on MS and WPM media, 0, 3, 4, 5, 6 mgL^{-1} BA, 0.2 mgL^{-1} $GA_{3,}$ and 0.1 mgL^{-1} IBAwere used. At the rooting stage, 0, 0.5, 1, 1.5, 2 mgL^{-1} IBA, 0.5 mgL^{-1} NAA, 0.2 mgL^{-1} GA_3 and 0.1 mgL^{-1} BA were added. The compositions of the plant growth regulators (PGRs) of the nutrient medium used are given in Table 1. A total of 30 gL^{-1} sugar was used in the nutrient media. The pH of the media was adjusted to 5.8, and 7 gL^{-1} agar was added. The prepared media were heated on a heater until boiling started. The mixture was then distributed into glass jars (75 ml/jar) and sterilized in an autoclave at 121 °C and 1.05 kg cm^{-2} pressure for 15 min.

Observations and measurements

The number of shoots and shoot lengths (mm) were taken in each 3 subculture. After the rooting stage, the plantlets were removed from the jar, the roots were washed, and after removing moisture with filter paper, the shoots and roots were cut and separated.

After recording the number of roots, root length (mm), root fresh weight (mg), and shoot fresh weight (mg), the materials were placed in an oven with compressed air circulation at 50 °C. Shoots and roots were dried for 120 h until a constant weight was achieved, and shoot dry weight and root dry weight were determined (Rodrigues et al. 2021).

Statistical analysis

All quantitative data expressed as percentages were subjected to arcsine transformation. The obtained data were analyzed using the JMP Software (Version 14.3, SAS Institute Inc., Cary, NC, USA) statistical program and analysis of variance was performed. The differences between means were separated using the LSD multiple comparison test at $p \leq 0.05$.

Results and discussion

During the shoot proliferation stage, 340 areoles were taken from each genotype, and an average of 86% of these areoles formed shoots. The healthiest 270 of the resulting shoots were selected and used in the propagation stage.

Number of shoots

Differences between nutrient media, BA concentrations and genotypes were found statistically significant. The highest average number of shoots (8.94) in explants cultured over 3 subcultures after the proliferation stage was obtained from *H. undatus* on MS medium containing 3 mgL^{-1} BA (Fig. 1). The highest propagation ratio (4.46) in *H. polyrhizus* was achieved on MS medium containing 5 mgL^{-1} BA (Fig. 2). *H. undatus* had a higher propagation rate (3.66) than *H. polyrhizus* (2.91). A higher average number of shoot (3.95) was obtained in the MS medium compared to the WPM medium (2.62 shoot/explant) (Table 2). The presence of cytokinins in the culture medium is essential for proliferation as the optimum cytokinin concentration stimulates the proliferation of shoots from the initial explants (Babaoğlu et al. 2001). The absence of BA slowed shoot propagation in both media. The optimum dose varied according to medium and genotypes.

Dahanayake and Ranawake (2011), reported obtaining three shoots per explant from *H. undatus* leaf explants on MS medium supplemented with 2.5 mgL^{-1} BA.Vinas et al., (2012) using 3 mgL^{-1} BAP in *H. costaricensis*, achieved a maximum 5 shoots per explant. Qin et al. (2017) obtained 6.4 shoot/explant from *H. polyrhizus* using 5.5 mgL^{-1} BA. Balch et al. (1998) also reported that the number of shoot varies according to genotype.



Fig. 1 Multiplicated *H. undatus* plantlets



Fig. 2 Multiplicated *H. polyrhizus* plantlets

Shoot length

Although the primary nutrient media were in the same statistical group regarding shoot length, the differences between BA concentrations and between varieties were statistically significant. *H. undatus* produced longer shoots (31.35 mm) than *H. polyrhizus* (28.81 mm). *H. undatus* formed the longest shoots (44.03 mm) in control WPM medium, while *H. polyrhizus* formed the longest shoots (41.83 mm) in control MS medium (Table 3).

IBA and NAA (auxins) are hormones that promote root formation and growth, but auxins can also affect shoot elongation. Auxins are generally not required during the shoot proliferation phase but are included in low concentrations to maintain balanced growth. Gibberellins are hormones that regulate stem elongation and plant growth. The balance between cytokinins and auxins is crucial to maintain normal growth and development of tissue culture plantlets. Appropriate hormone ratios also ensure balanced vegetative growth (Babaoğlu et al. 2001).

Bozkurt et al. (2022) reported a shoot length of 42.8 mm in the Royal Red variety in medium containing 1 mgL-1 BA. Usta (2022) found shoot lengths of 12 mm in the medium containing 1 mgL^{-1} KIN and 11 mm in the medium containing 2 mgL^{-1} BA for *H. undatus*. Mohamed-Yasseen (2002) obtained a shoot length of 33 mm in *H. undatus* in a medium containing 0.01 µM TDZ.

Table 2 Effects of different media, genotypes and BA (mgL^{-1}) doses on the number of shoots

Genotypes	Medium	Control	3.0 BA	4.0 BA	5.0 BA	6.0 BA	Medium average	Genotype average
H. undatus	MS	3.03 (10.03) ef	8.94 (17.40) a	3.42 (10.65) cde	3.60 (10.93) c	3.27 (10.41) cde	MS 3.95 (11.25) A	3.66 (10.80) A
	WPM	2.47 (9.03) hıj	3.17 (10.22)def	2.82 (9.67) fgh	3.29 (10.46) cde	2.59 (9.22) ghı		
H. polyrhizus	MS	2.19 (8.51) j	4.24 (11.87) b	2.84 (9.69) fg	4.46 (12.19) b	3.49 (10.77) cd	WPM 2.62 (9.21) B	2.91 (9.66) B
	WPM	2.25 (8.62) ıj	1.58 (7.21) k	3.17(10.22) def	3.57 (10.89) c	1.32 (6.59) k		
BA average		**2.49 (9.05) D**	**4.48 (11.67) A**	**3.06 (10.06) C**	**3.73 (11.12) B**	**2.67 (9.25) D**		

LSD medium: 0.203***, LSD genotype: 0.203***, LSD medium*genotype: NS, LSD BA: 0.320***, LSD genotype*BA: 0.454***, LSD medium*BA: 0.454***, LSD medium*genotype*BA: 0.642***

Values are given in bold and underlined to distinguish statistical results of different parameters

NS non-significant

There is a statistically significant difference ($P<0.01$**, $P<0.001$***) between the groups indicated with different letters

Table 3 Effects of different media, genotypes and BA (mgL^{-1}) doses on shoot length (mm)

Genotypes	Medium	Control	3.0 BA	4.0 BA	5.0 BA	6.0 BA	Medium average	Genotype average
H. undatus	MS	36.93 d	37.73 cd	27.83 gh	29.26 fg	33.50 e	MS 30.30	31.35 A
	WPM	44.03 a	25.27 ı	22.00 k	27.90 fgh	29.03 fg		
H. polyrhizus	MS	41.83 b	21.13 k	21.90 k	26.70 hı	26.20 ı	WPM 29.85	28.81 B
	WPM	38.80 c	25.27 ı	22.00 k	27.90 fgh	29.03 fg		
BA average		**40.40 A**	**29.28 B**	**24.30 D**	**26.89 C**	**29.51 B**		

LSD medium: NS, LSD genotype: 0.474***, LSD medium*genotype: 0.670***, LSD BA: 0.749***, LSD genotype*BA: 1.060***, LSD medium*BA: 1.060***, LSD medium*genotype*BA: 1.499***

Values are given in bold and underlined to distinguish statistical results of different parameters

NS non-significant

There is a statistically significant difference ($P<0.01$**, $P<0.001$***) between the groups indicated with different letters

Number of roots

Differences between basic nutrient media, IBA concentrations, and varieties in terms of average root number were found statistically significant. The highest number of roots (4.77 roots/shoot) was obtained from *H. undatus* in WPM medium containing 2 mgL^{-1} IBA (Fig. 3). The WPM medium, IBA doses ranging from 0.5 to 2.0 mgL^{-1}, and *H. polyrhizus* genotype had higher average values regarding rooting (Table 4). IBA is critical for promoting root formation in tissue culture plantlets. The optimum auxin doses that promote high root formation and elongation, leading to the development of a robust root system varied significantly depending on the primary nutrient medium and genotype (Babaoğlu et al. 2001). Qin et al. (2017), reported 7.21 roots/shoot in *H. polyrhizus* using a medium containing 0.3 mgL^{-1} IBA while Deng et al. (2019) obtained 7.3 roots/shoot in a medium containing 0.2 mgL^{-1} NAA.



Fig. 3 *H. undatus* plantlets 5 weeks after transferring to root medium

Table 4 Effects of different media, genotypes and IBA (mgL^{-1}) doses on the number of roots

Genotypes	Medium	Control	0.5 IBA	1.0 IBA	1.5 IBA	2.0 IBA	Medium average	Genotype average
H. undatus	MS	2.03 (8.20) jk	2.11 (8.35) j	2.29 (8.71) ı	2.59 (9.25) fgh	2.59 (9.25) fgh	MS 2.55 (9.16) B	2.70 (9.37) B
	WPM	1.88 (7.88) k	3.11 (10.15) e	3.00 (9.97) e	2.66 (9.39) fg	4.77 (12.57) a		
H. polyrhizus	MS	2.77 (9.58) f	3.77 (11.20) c	2.48 (9.05) ghı	2.44 (8.98) hı	2.44 (8.98) hı	WPM 3.08 (9.94) A	2.93 (9.73) A
	WPM	1.44 (6.89) l	4.33 (12.01) b	4.44 (12.16) b	1.88 (7.88) k	3.33 (10.51) d		
IBA average		**2.03 (8.14) B**	**3.33 (10.43) A**	**3.05 (9.97) A**	**2.39 (8.88) B**	**3.27 (10.33) A**		

LSD medium: 0.110***, LSD genotype: 0.110***, LSD medium*genotype: 0.156***, LSD IBA: 1.019***, LSD genotype*IBA: 0.246***, LSD medium*IBA:0.246***, LSD medium*genotype*IBA: 0.348***

Values are given in bold and underlined to distinguish statistical results of different parameters

There is a statistically significant difference (P < 0.01**, P < 0.001***) between the groups indicated with different letters

Root length

Differences in root length were statistically significant between nutrient medium, IBA concentrations, and genotypes. Although the highest average root length (67.42 mm) was obtained from *H. undatus* in WPM medium containing 0.5 mgL^{-1} IBA, the MS medium, and *H. polyrhizus* showed better average values overall (Table 5) (Fig. 4). Usta (2022) reported a root length of 15 mm in a medium containing 1 mgL^{-1} IBA. Qin et al. (2017) found a root length of 27.3 mm in a medium containing 0.3 mgL^{-1}. Deng et al. (2019) obtained a root length of 90 mm in a medium containing 0.1 mgL^{-1} NAA.

Shoot fresh weight

Differences between nutrient media, IBA concentrations, and varieties were found statistically significant in terms of average shoot fresh weight. The highest average shoot fresh weight (1183.33 mg) was obtained from *H. undatus* cultered in the WPM containing 2.0 mgL^{-1} IBA. *H. polyrhizus* (641.97 mg), *H. undatus* (570.23 mg); WPM medium (721.40 mg) to MS medium (490.80 mg); The 2.0 mgL^{-1} IBA concentration (795.03 mg) had a higher average shoot fresh weight than other IBA concentrations (Table 6). Dewir et al. (2023) measured the shoot fresh weight of *H. polyrhizus* as 872 mg in the medium containing 1 mgL^{-1} BAP, while Bhanwariya et al. (2023) measured it as 600 mg in 0.3 µM NAA.

Shoot dry weight

Differences between nutrient media, IBA concentrations, and varieties were found to be statistically significant in terms of average shoot dry weight. The highest average shoot dry weight (106.0 mg) was obtained from *H. undatus* cultured in the WPM medium, in the medium containing 2.0 mgL^{-1} IBA (Fig. 5). *H. polyrhizus* had an average shoot dry weight of 52.90 mg, compared to 46.47 mg for *H. undatus*. The WPM medium (57.77 mg) resulted in a higher average shoot dry weight than the MS medium (41.60 mg). The 2.0 mg/L IBA concentration (66.08 mg) produced a higher average shoot dry weight than other IBA concentrations (Table 7). Dewir et al. (2023) determined the shoot dry weight of H. polyrhizus to be 120 mg in medium containing 1 mg/L BAP.

Table 5 Effects of different media, genotypes and IBA (mgL^{-1}) doses on root length (mm)

Genotypes	Medium	Control	0.5 IBA	1.0 IBA	1.5 IBA	2.0 IBA	Medium average	Genotype average
H. undatus	MS	52.34 ef	52.25 ef	41.98 h	46.21 g	58.17 c	MS 51.22 A	49.59 B
	WPM	62.14 b	67.42 a	31.84 k	38.47 j	45.04 g		
H. polyrhizus	MS	64.40 b	57.43 cd	45.57 g	38.91 ıj	54.96 de	WPM 49.99 B	51.63 A
	WPM	41.32 hı	50.49 f	56.38 cd	49.94 f	56.87 cd		
IBA average		**55.05 B**	**56.90 A**	**43.94 C**	**43.38 C**	**53.76 B**		

LSD medium: 0.899**, LSD genotype: 0.899***, LSD medium*genotype: NS, LSD IBA: 1.423***, LSD genotype*IBA: 2.012***, LSD medium*IBA: 2.012***, LSD medium*genotype*IBA: 2.846***

Values are given in bold and underlined to distinguish statistical results of different parameters

NS non-significant

There is a statistically significant difference (P < 0.01**, P < 0.001***) between the groups indicated with different letters



Fig. 4 Appearance of root length in the medium

Table 6 Effects of different media, genotypes and IBA (mgL^{-1}) doses on shoot fresh weight (mg)

Genotypes	Medium	Control	0.5 IBA	1.0 IBA	1.5 IBA	2.0 IBA	Medium average	Genotype average
H. undatus	MS	462.00 ıj	346.67 kl	333.33 l	360.00 jkl	459.67 ıj	MS 490.80 B	570.23 B
	WPM	554.33 ghı	771.33 de	590.33 gh	641.33 fg	1183.33 a		
H. polyrhizus	MS	741.67 ef	806.67 cde	370.33 jkl	527.00 hı	500.67 hı	WPM 721.40 A	641.97 A
	WPM	209.67 m	861.33 cd	909.00 c	456.67 ıjk	1036.67 b		
IBA average		**491.92 D**	**696.50 B**	**550.75 C**	**496.25 CD**	**795.03 A**		

LSD medium: 35.196***, LSD genotype: 35.196***, LSD medium*genotype: 49.775***, LSD IBA:55.650***,LSD genotype*IBA: 78.701***, LSD medium*IBA: 78.701***, LSD medium*genotype*IBA: 111.300***

Values are given in bold and underlined to distinguish statistical results of different parameters

There is a statistically significant difference ($P<0.01$**, $P<0.001$***) between the groups indicated with different letters

Root fresh weight

Differences between nutrient media, IBA concentrations, and varieties were found statistically significant in terms of average root fresh weight. The highest average root fresh weight (838.67 mg) was obtained in *H. polyrhizus*, cultured in the the WPM medium containing 1.0 mgL^{-1} IBA. Regarding IBA concentrations, the highest average root fresh weight value (604.83 mg) was obtained from 2.0 mgL^{-1} IBA. The average root fresh weight was 275.60 mg for *H. undatus* and 399.97 mg for *H. polyrhizus* (Table 8).

Root dry weight

Differences between nutrient media, IBA concentrations, and varieties were found statistically significant in terms of average root dry weight. The highest average root dry weight (76.0 mg) was recorded in *H. polyrhizus* cultured in WPM medium containing 1.0 mg/L IBA. Regarding IBA concentrations, the highest average root dry weight (49.75 mg) was achieved with 2.0 mg/L IBA (Table 9) (Fig. 6).

Conclusion

Understanding the interactions between hormones that regulate various physiological processes in plants and optimizing their concentrations accordingly is essential for successful in vitro propagation of *Hylocereus* spp. It has been determined that the growth regulator compound used in the basic nutrient medium in micropropagation of *Hylocereus* species has significant effects on shoot initiation, proliferation, rooting, and the growth and development of plantlets. Optimum hormone concentrations varied depending on the specific species of *Hylocereus* and the tissue culture stage. In in vitro propagation of *Hylocereus* species, it is

Fig. 5 Dried shoots



Table 7 Effects of different media, genotypes and IBA (mgL^{-1}) doses on shoot dry weight (mg)

Genotypes	Medium	Control	0.5 IBA	1.0 IBA	1.5 IBA	2.0 IBA	Medium average	Genotype average
H. undatus	MS	27.33 lm	32.33 kl	35.00 ıjkl	39.00 hıjk	33.33 jkl	MS 41.60 B	46.47 B
	WPM	38.33 ıjk	61.67 cd	48.67 fgh	43.00 ghıj	106.00 a		
H. polyrhizus	MS	51.00 efg	67.33 cd	33.33 jkl	58.33 def	39.00 hıjk	WPM 57.77 A	52.90 A
	WPM	19.33 m	59.33 de	71.00 c	44.33 ghı	86.00 b		
IBA average		**34.00 D**	**55.17 B**	**47.00 C**	**46.17 C**	**66.08 A**		

LSD medium: 3.217***, LSD genotype: 3.217***, LSD medium*genotype: 4.549***, LSD IBA: 5.086***, LSD genotype*IBA: 7.193***, LSD medium*IBA: 7.193***, LSD medium*genotype*IBA: 30.518***

Values are given in bold and underlined to distinguish statistical results of different parameters

There is a statistically significant difference ($P<0.01$**, $P<0.001$***) between the groups indicated with different letters

Table 8 Effects of different media, genotypes and IBA (mgL^{-1}) doses on root fresh weight (mg)

Genotypes	Medium	Control	0.5 IBA	1.0 IBA	1.5 IBA	2.0 IBA	Medium average	Genotype average
H. undatus	MS	47.33 k	244.33 ghı	102.33 jk	274.67 fg	364.67 ef	MS 252.93 B	275.60 B
	WPM	69.67 k	401.00 de	261.33 ghı	171.33 ıj	819.33 a		
H. polyrhizus	MS	102.33 jk	522.00 bc	177.00 hıj	229.33 ghı	465.33 cd	WPM 422.63 A	399.97 A
	WPM	20.33 k	586.67 b	838.67 a	288.00 fg	770.00 a		
IBA average		**59.92 E**	**438.50 B**	**344.83 C**	**240.83 D**	**604.83 A**		

LSD medium: 30.917***, LSD genotype: 30.917***, LSD medium*genotype: 43.723*, LSD IBA: 48.883***, LSD genotype*IBA: 69.132***, LSD medium*IBA: 69.132***, LSD medium*genotype*IBA: 97.767***

Values are given in bold and underlined to distinguish statistical results of different parameters

There is a statistically significant difference ($P<0.01$**, $P<0.001$***) between the groups indicated with different letters

important to optimize the concentrations of growth regulators and other components according to the specific requirements and responses of the propagated species. Developing effective micropropagation protocols will enable the rapid propagation of elite clones and the production of healthy propagation material.

This study was carried out to develop a protocol for micropropagation and rooting of *H. undatus* and *H.*

Table 9 Effects of different media, genotypes and IBA (mgL^{-1}) doses on root dry weight (mg)

Genotypes	Medium	Control	0.5 IBA	1.0 IBA	1.5 IBA	2.0 IBA	Medium average	Genotype average
H. undatus	MS	8.33 hı	26.67 de	15.33 gh	21.33 efg	32.67 d	MS 24.70 B	27.07 B
	WPM	6.33 ı	45.00 c	28.33 de	18.33 fg	68.33 ab		
H. polyrhizus	MS	14.33 gh	48.67 c	21.67 efg	23.67 ef	34.33 d	WPM 38.30 A	35.93 A
	WPM	2.67 ı	50.67 c	76.00 a	23.67 ef	63.67 b		
IBA average		**7.91 E**	**42.75 B**	**35.33 C**	**21.75 D**	**49.75 A**		

LSD medium: 2.437***, LSD genotype: 2.437***, LSD medium*genotype: NS, LSD IBA: 3.852***, LSD genotype*IBA: 5.448***, LSD medium*IBA: 5.448***, LSD medium*genotype*IBA: 7.705***

Values are given in bold and underlined to distinguish statistical results of different parameters

NS non-significant

There is a statistically significant difference ($P < 0.01$**, $P < 0.001$***) between the groups indicated with different letters



Fig. 6 Dried roots

polyrhizus pitaya species in different basic nutrient media containing different doses of plant growth regulators. At the propagation stage, the MS medium and 3 mgL^{-1} BA doses were determined as the best combination for both species. *H. undatus* produced the longest shoots in without hormone MS and *H. polyrhizus* without hormone WPM medium. WPM medium gave better results than MS medium in terms of root number, root length, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight. Studies on pitaya are still very limited; only MS medium was used in all of them. However, tissue culture studies, which yield different results depending on the species and even the variety, need to be enriched. Thus, the most suitable nutrient medium and growth regulator combinations can be obtained at the variety level.

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Data availability The author confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

Declarations

Conflict of interest The author declare that there are no conflicts of interest related to this article. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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