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Influence of root inducers and substrates on the vegetative propagation of pitahaya species (*Hylocereus* spp.) under nursery conditions

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Abstract

Pitahaya (*Hylocereus* spp.) is an emerging crop for tropical regions; however, its vegetative propagation is strongly influenced by environmental and management factors. Optimizing rooting and establishment under nursery conditions is essential to produce uniform and vigorous seedlings. This study evaluated the effects of root inducers and substrates on the vegetative propagation of three *Hylocereus* species under nursery conditions. A completely randomized design (CRD) with a 2×2×3 factorial arrangement was implemented, including two root inducers (NAA + IBA solution and seaweed extract), two substrates (substrate 1 and substrate 2), and three species (*H. undatus*, *H. megalanthus*, and *H. costaricensis*), resulting in 12 treatments with four replicates each. Seven variables were assessed: shoot emission time (SET), cutting viability (CV), number of shoots (NS), shoot length (SL), number of roots (NR), root length (RL), and vegetative vigor index (VVI). ANOVA revealed significant effects ($p \leq 0.05$) in the three-factor interaction for SET, CV, NS, SL, RL, and VVI. Treatment T9 achieved the shortest SET (21.53 days) and highest CV (100%), T7 recorded the highest NS (1.62 shoots plant^{-1}), T3 showed greater SL (68.67 cm) and NR (5.30 roots plant^{-1}), and T12 obtained the highest RL (42.69 cm) and VVI (110.18). Effect size analysis based on partial eta squared (ηp^2) identified species as the most influential factor, explaining 55–98% of the observed variance. Among the evaluated species, *H. costaricensis* showed the best overall performance. These findings demonstrate that combining *H. costaricensis*, substrate 1, and NAA + IBA enhances vegetative propagation efficiency. These findings provide valuable evidence for improving pitahaya propagation protocols and optimizing seedling production under nursery conditions.

Key words: Phytohormones, cuttings, rooting, organic substrate, *Hylocereus* spp.

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1. Introduction

Pitahaya (*Hylocereus* spp.), commonly known as dragon fruit, is a cactus belonging to the genus *Hylocereus* within the family Cactaceae. The genus comprises approximately 14 species, of which *H. undatus*, *H. monacanthus* (Lem.) Britton & Rose, *H. costaricensis*, and *H. megalanthus* are among the most widely cultivated worldwide [1,2]. Species of this genus are native to tropical and subtropical regions of the Americas [3], and have high export potential because of their nutritional value and adaptability to a wide range of soil and climatic conditions [4]

Global pitahaya production has increased steadily, with Vietnam, China, and Indonesia together accounting for nearly 93% of total global output [5]. In Latin America, pitahaya has gained importance as an alternative crop that improves rural livelihoods and contributes to agricultural sustainability and diversification [4]. In Peru, the cultivated area expanded markedly from 34.2 ha in 2019 to 419.2 ha in 2023, accompanied by a rapid increase in production from 156.9 t to 4,178.9 t [6] . At the regional level, Lima and Piura lead national production, accounting for 48.4% and 36.1% of total output, respectively, followed by Ica, Amazonas, Moquegua, and La Libertad [6]. These trends indicate growing interest in pitahaya cultivation and its potential as a sustainable and commercially viable crop. However, the rapid expansion of cultivated areas also poses new challenges, particularly regarding the availability of high-quality genetic material to support newly established plantations [7].

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Although pitahaya can be propagated by seed, this approach does not ensure genetic uniformity. Therefore, vegetative propagation through stem cuttings is preferred as a more efficient approach [8]. This method is widely used because vegetatively propagated plants exhibit faster growth, earlier fruiting, and greater genetic stability [9,10]. Achieving effective vegetative propagation depends on several key factors that play a crucial role during the rooting stage [11]. Among these, the application of auxins and the selection of an appropriate rooting substrate are considered critical [12,13]. Understanding and optimizing these factors are essential to ensure successful propagation and enhance productivity at the commercial scale.

Previous studies have shown that the choice of rooting agent, whether synthetic or organic, has a significant effect on the formation of adventitious roots in cuttings [14], as well as root length and shoot development in vegetatively propagated species [10,11,15,16]. Among synthetic rooting agents, the most widely used auxins across a wide range of plant species are indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA), which promote cell division and differentiation, although their effectiveness depends on concentration and plant species [11,17]. Moreover, seaweed extracts derived from *Ecklonia maxima* contain phytohormones such as auxins, cytokinins, ethylene, and gibberellins, which can stimulate root formation and overall plant growth [18–20]. These extracts have also been reported to improve soil health, plant growth, and stress tolerance through the action of natural phytohormones, amino acids, polysaccharides, and micronutrients [21,22]. Accordingly, comparing synthetic and organic rooting agents is essential for developing efficient and sustainable propagation strategies for pitahaya.

In addition, substrate composition is a key factor influencing the success of cutting establishment, as it is essential for moisture retention and nutrient availability during the rooting process [15]. One of the most suitable substrates can be formulated by combining organic components such as sawdust, peat, rice husks, and cattle manure, which together provide optimal physical and chemical conditions for root development and cutting establishment [23–25].

Given the increasing commercial demand for pitahaya and the limited technical information available on its efficient propagation in tropical regions, there is a need for studies aimed at optimizing seedling production. In this context, the present study evaluated the effect of two types of rooting agents and two types of substrates on the vegetative propagation of three *Hylocereus* spp. species in a controlled nursery environment. We hypothesized that an appropriate combination of rooting agents and substrates would significantly improve rooting success and promote the development of vigorous and high-quality pitahaya seedlings.

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2. Methodology

2.1. Study area

The study was conducted at the Center for Plant Production for Research and Technology Transfer, Centro Experimental Yanayacu, Instituto Nacional de Innovación Agraria (INIA), located in the province of Jaén, Cajamarca Department, Peru (5.6771° S, 78.7749° W), at an elevation of 624 m a.s.l. (Supplementary Figure S1). During the experimental period, the mean temperature, accumulated precipitation, and relative humidity were 26.96 °C, 175 mm, and 45.73%, respectively [26].

2.2. Biological material

Three *Hylocereus* species were evaluated (Figure 1): two red-skinned species, *H. undatus* and *H. costaricensis*, collected from mature pitahaya plantations at Grupo Campo Agro S.A., in the province of Zarumilla, Tumbes department; and one yellow-skinned species, *H. megalanthus*, sourced from plantations in the province of San Ignacio. Species selection was driven by their adaptability to tropical environments, favorable agronomic and organoleptic traits, and strong commercial demand in both domestic and international markets.

2.3. Experimental design

The experiment was conducted using a completely randomized design (CRD) with a 2A × 2B × 3C factorial arrangement, consisting of two rooting inducers (seaweed extract and a solution of NAA and IBA), two substrate types (substrate 1 and substrate 2), and three pitahaya species (*H. undatus*, *H. costaricensis*, and *H. megalanthus*). The combination of factor levels resulted in 12 treatments. Each treatment included four independent replicates, and each replicate consisted of ten cuttings, resulting in 48 experimental units and 480 cuttings in total. For statistical analysis, the mean value of the ten cuttings within each replicate was used as the observational unit; therefore, the ANOVA was performed with four independent observations per treatment, avoiding pseudoreplication.

Table 1. Description of treatments and interaction of factors.

Treatment	Combination of factors		
	Rooting agent	**Substrate**	**Species**
1	Seaweed	Substrate 1	*H. undatus*
2	Seaweed	Substrate 1	*H. megalanthus*
3	Seaweed	Substrate 1	*H. costaricensis*
4	Seaweed	Substrate 2	*H. undatus*
5	Seaweed	Substrate 2	*H. megalanthus*
6	Seaweed	Substrate 2	*H. costaricensis*
7	NAA + IBA	Substrate 2	*H. undatus*
8	NAA + IBA	Substrate 2	*H. megalanthus*
9	NAA + IBA	Substrate 2	*H. costaricensis*
10	NAA + IBA	Substrate 1	*H. undatus*
11	NAA + IBA	Substrate 1	*H. megalanthus*
12	NAA + IBA	Substrate 1	*H. costaricensis*

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2.4. Setting up the experiment

2.4.1. Preparation of vegetative seed

Cuttings were obtained from healthy, pest-free mother plants at the productive stage (Figure 1). Lignified cladodes were selected, and 40 cm segments were taken from the central portion, avoiding both the young apical region and the senescent basal end. Thus, vegetative material was collected from *H. undatus*, *H. megalanthus*, and *H. costaricensis* (Figure 1). At the basal end of each cutting, a V-shaped incision was made to expose 5–7 cm of the vascular tissue. The cuttings were disinfected by immersion in a copper (II) sulfate pentahydrate ($CuSO_4 5H_2O$) solution for 5 min and subsequently allowed to cure for 5 days in a dry, shaded environment under diffuse light.

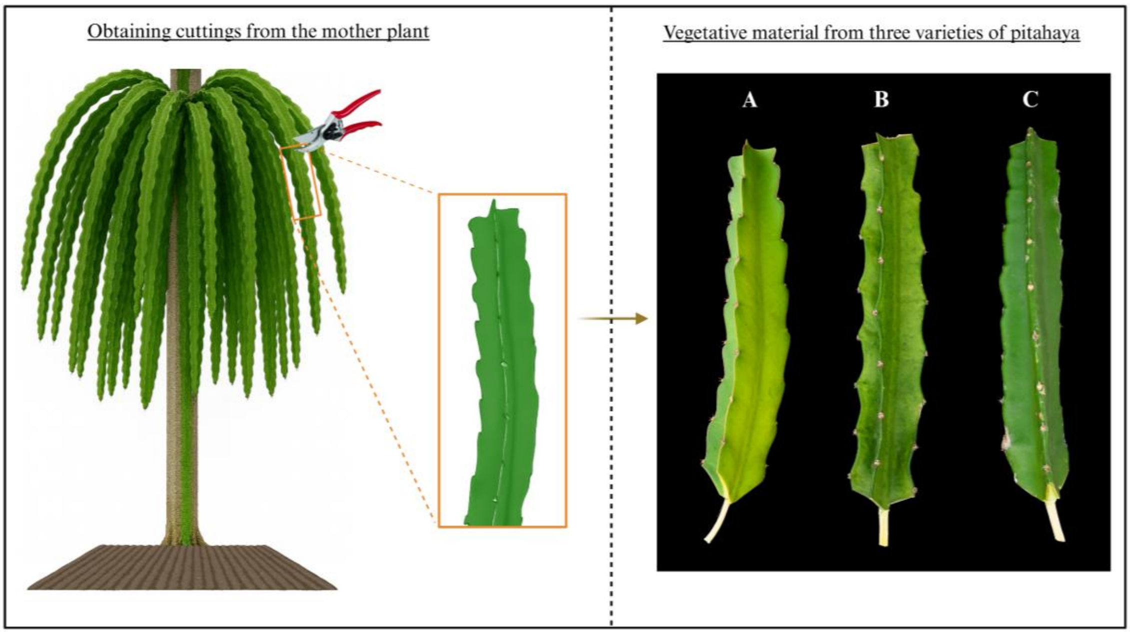

Figure 1. Obtaining cuttings from a mother plant and vegetative material from three species of pitahaya. A) *H. undatus*, B) *H. megalanthus* and C) *H. costaricensis*.

2.4.2. Preparation of substrates

The substrates used in the experiment differed in composition. Substrate 1 consisted of forest soil, compost, and cattle manure mixed in a 3:4:3 ratio, whereas Substrate 2 was composed of river sand and forest soil in a 4:6 ratio. Prior to use, the physicochemical properties of both substrates, including pH, electrical conductivity, organic matter, total nitrogen, available phosphorus, calcium carbonate equivalent, and particle-size distribution, were determined. Detailed substrate characteristics are presented in Supplementary Table S5.

Both substrates were sieved and sterilized using the solarization method with low-density polyethylene (LDPE) plastic sheets, which allow solar radiation to penetrate while trapping heat within the substrate, thereby disrupting the life cycle of soil-borne pathogenic organisms [27]. Following sterilization, the substrates were placed in 10 × 25 cm polyethylene bags with gussets and arranged according to the corresponding treatments and replicates.

2.4.3. Application of rooting inducers

Two rooting agents were used: an *Ecklonia maxima*–based seaweed extract as an organic rooting agent (15 mL L^{-1}) and a synthetic rooting agent containing 1-naphthaleneacetic acid (NAA, 0.40%) and indole-3-butyric acid (IBA, 0.10%). The product was applied at a concentration of 2 mL L^{-1}, resulting in final concentrations of 8 mg L^{-1} NAA (42.96 μM) and 2 mg L^{-1} IBA (9.84 μM), corresponding to a 4:1 NAA:IBA ratio. Both products function as biostimulants by modulating plant physiological responses [28,29]. Application was performed by immersing the basal end of each cutting in the respective solution for 10 min in the case of the seaweed extract and for 2 min in the case of the NAA + IBA solution prior to planting.

2.4.4. Planting and maintenance

The basal end of each cutting was inserted into polyethylene bags containing sterilized substrate until the V-shaped incision was completely covered. The substrate was lightly compacted to ensure stability the cutting. All bags were labeled and arranged according to the experimental design and replicates. Plants were maintained in a nursery under a Raschel shade net providing 60% shade throughout the evaluation period. Irrigation was applied as needed to maintain adequate substrate moisture throughout the experiment. Routine maintenance practices, including weed removal and insect pest control, were performed manually, while fungal diseases were prevented through periodic applications of copper (II) sulfate pentahydrate ($CuSO_4 5H_2O$).

2.5. Parameters evaluated

Six variables were evaluated during the four months following planting: shoot emission time, cutting viability, number of shoots, shoot length, number of roots, and root length. In addition, a composite variable, termed the Vegetative Vigor Index, was calculated by integrating all evaluated variables except time to shoot emergence, due to its inverse nature. The evaluation period was defined based on the growth dynamics of the studied

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Hylocereus species and extended until the plants reached an optimal size for transplantation to the final field.

- *Shoot emergence time (SET)*

This variable was assessed as the number of days from planting to the emergence of the first visible vegetative shoot (≥ 3 mm) from an areole of the cladode.

- *Cutting viability (CV)*

The percentage of viable cuttings was determined by classifying cuttings that developed at least one shoot by the end of the nursery stage as viable. The calculation was performed using the formula adapted from Jose & Sivaprasad [30].

$$VE = \frac{Cuttingswith \geq 1vegetativeshoot}{Totalnumber\ of\ cuttings\ evaluated} x100 \quad (1)$$

- *Number of shoots (NS)*

This variable was assessed by counting the total number of visible vegetative shoots (≥ 3 mm) per cutting. Evaluations were conducted at 15-day intervals, and the value recorded at the final assessment was used for analysis. Dormant buds and senescent shoots were excluded from this variable.

- *Shoot length (SL)*

Shoot length was measured using a measuring tape graduated in centimeters, from the point of insertion on the cutting to the shoot apex. When a cutting developed a single shoot, its individual length was recorded. In cuttings with multiple shoots, shoot length was calculated as the average length of all developed shoots.

- *Number of roots (NR)*

The number of roots per cutting was recorded at the end of the nursery stage. For this assessment, the substrate was carefully removed, and the root system was gently washed several times to avoid mechanical damage. Only roots emerging directly from the vascular bundle were counted.

- *Root length (RL)*

The substrate was carefully removed from the root zone, and root length was measured using a tape measure graduated in centimeters, from the point of root emergence to the tip of the longest root.

- *Vegetative vigor index (VVI)*

The Vegetative Vigor Index (VVI) was calculated using the formula adapted from BharathKumar et al.[31] and Fetouh & Hassan [32].

$$VVI = \frac{(CV) * (NS + SL + NR + RL)}{100} \quad (2)$$

Where CV is cutting viability (%), NS is the number of shoots, SL is shoot length (cm), NR is the number of roots, and RL is root length (cm). SET was not included in the equation because it is a temporal variable for which lower values indicate better performance. This interpretation is opposite to that of the other variables included in the

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index, where higher values reflect greater vigor. Therefore, its direct inclusion in the equation would be inconsistent with the structure of the VVI.

2.6. Statistical analysis

Statistical analyses were conducted using R software version 4.4.3 [33] with the packages dplyr [34], agricolae [35], ggplot2 [36], and corrplot [37]. All variables, except Cutting Viability (CV), were tested for the normality of residuals using the Shapiro Wilk test ($p \leq 0.05$) and for homogeneity of variances using Levene's test. A three-way analysis of variance (ANOVA; $p \leq 0.05$) was performed to evaluate the effects of rooting agents, substrates, and pitahaya species, and mean comparisons were conducted using Tukey's test. Because CV represents a proportion derived from counts of viable and non-viable cuttings, it was analyzed using a generalized linear model (GLM) with binomial error distribution and logit link function. In addition, a Principal Component Analysis (PCA) and a Spearman correlation test were carried out to assess the relationships among the evaluated variables.

3. Results

3.1. Establishment

Shoot emission time (SET) and cutting viability (CV) were evaluated during the establishment stage.

The three-way ANOVA (A × B × C) indicated significant differences among treatments for both SET and CV ($p < 0.05$), with coefficients of variation of 5.40% and 16.43%, respectively (Supplementary Table S1). For SET, the shortest shoot emission times were observed in treatments T9, T3, T12, and T6 (group d), with mean values of 21.53, 23.31, 23.43, and 24.71 days, respectively (Figure 2A). In contrast, treatments T2 and T8 (group a) exhibited the longest emission times, averaging 68.19 and 67.87 days. The remaining treatments showed intermediate SET values, forming groups ab, bc, and c.

For cutting viability, the binomial GLM revealed significant differences among treatments (Supplementary Table 2, Figure 2B). Treatments T3, T9, and T12 achieved the highest viability values, all reaching 100%, whereasT8 and T2 showed the lowest viability percentages, with mean values of 40% and 42.5%, respectively. The remaining treatments, showed intermediate viability levels ranging between 60% and 95%.

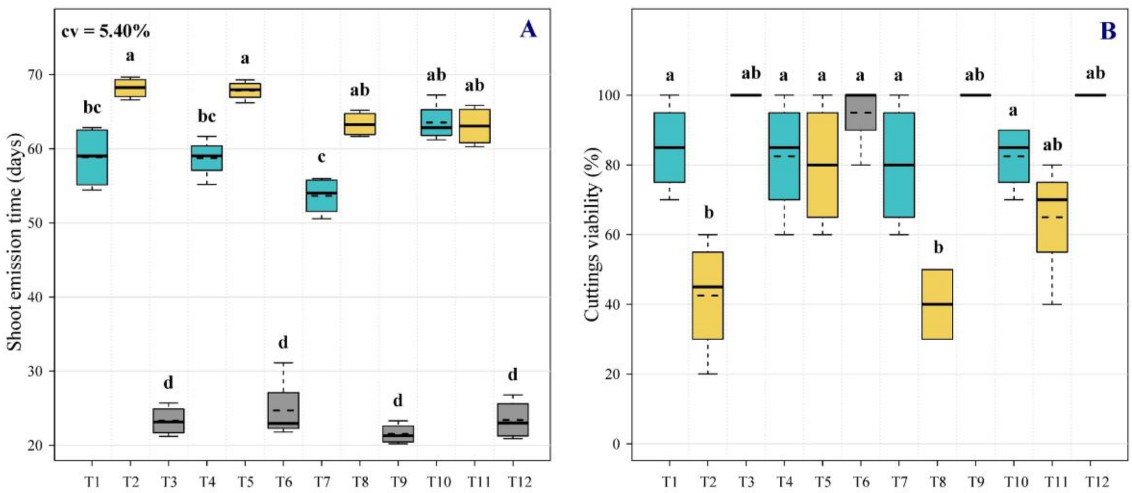

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Figure 2. Effects of 12 treatments (A × B × C) on cutting establishment variables. (A) Shoot emission time (SET) and (B) percentage of cutting viability (CV, %). Different letters above the bars indicate statistically significant differences according to Tukey's test for SET and binomial GLM pairwise comparisons for CV ($p \leq 0.05$).

Figure 3 illustrates the response of pitahaya species to the interaction of rooting agents and substrates on shoot emission time (SET) and cutting viability (CV). For SET, the interaction between rooting agents and species (A × C) was not statistically significant ($p = 0.0518$), although differences in mean values were observed among treatment combinations (Figure 3A). The shortest SET was observed in *H. costaricensis* treated with NAA + IBA, with a mean value of 22.48 days, whereas the longest SET corresponded to *H. megalanthus* treated with the seaweed extract, averaging 68.03 days. *H. undatus* under both rooting agents showed intermediate SET values (Figure 3A). In contrast, the substrate × species interaction (B × C) significantly affected SET ($p = 0.0184$) (Figure 3B). Tukey's test separated three distinct groups, with the shortest SET recorded in *H. costaricensis* grown in substrate 2 (23.12 days). Conversely, the longest SET was observed in *H. megalanthus* grown in substrate 1 (65.63 days), while *H. undatus* exhibited intermediate SET values under both substrates.

Figure 3C shows cutting viability (CV) as affected by the interaction between rooting agents and species (A × C), which was not statistically significant. Nevertheless, differences in mean viability values were observed among treatment combinations. *H. costaricensis* exhibited the highest viability values under both rooting agents, reaching 100% and 97.5% for NAA + IBA and seaweed extract, respectively. In contrast, *H. megalanthus* exhibited the lowest viability under NAA + IBA and seaweed extract treatments, with mean values of 52.5% and 61.25%, respectively. *H. undatus* showed intermediate CV values under both rooting agents. Likewise, Figure 3D presents cutting viability (CV) in relation to the substrate × species interaction (B × C), which was also not statistically significant. However, differences in mean viability values were observed among species and substrate combinations. *H. costaricensis* achieved the highest viability when grown in substrates 1 and 2, with values of 100% and 97.5%, respectively. Conversely, *H. megalanthus* showed the lowest viability in substrates 1 and 2, reaching 53.75% and 60%, respectively, whereas *H. undatus* displayed intermediate CV values under both substrates.

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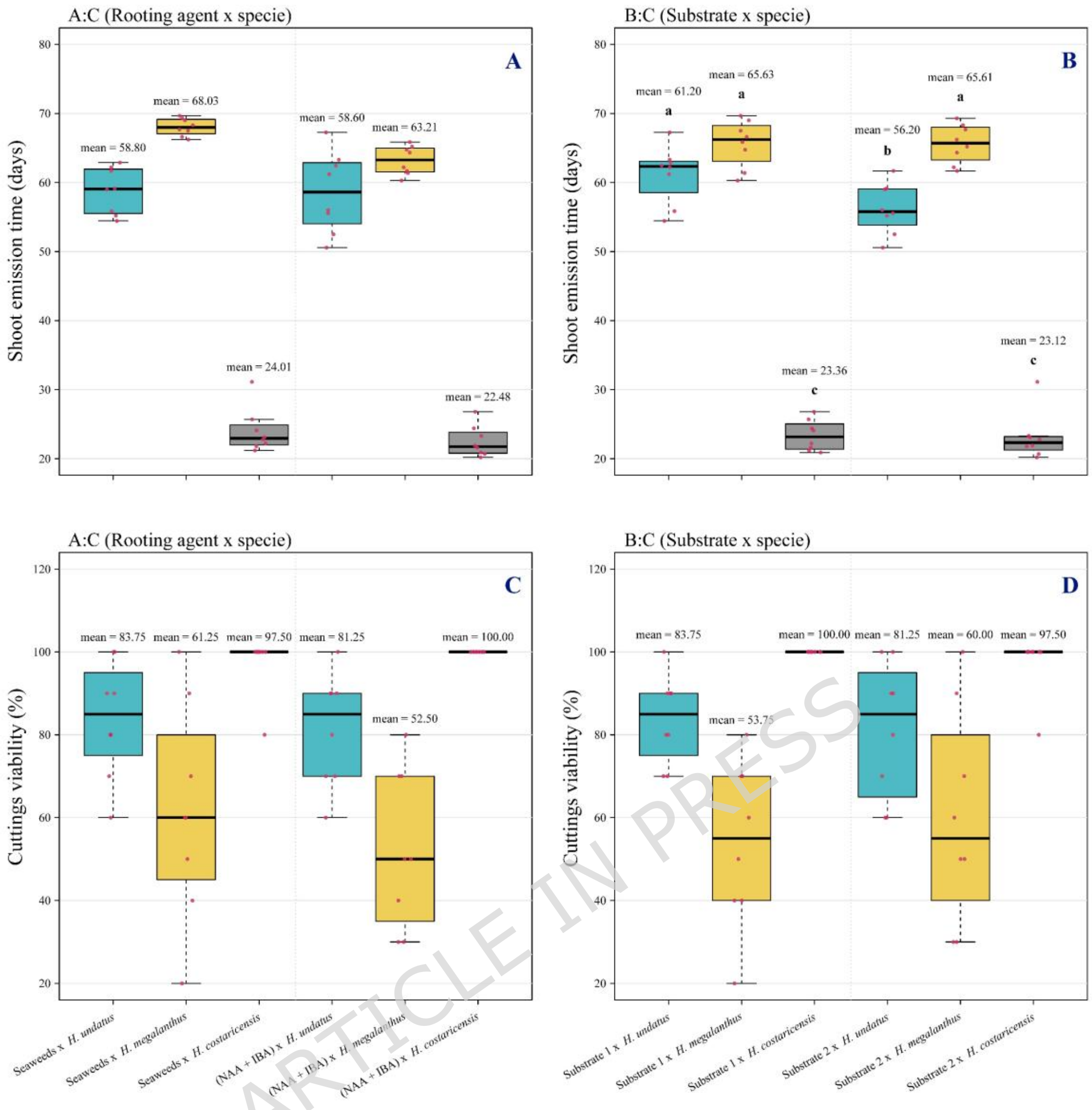

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Figure 3. Impact of rooting agents and substrates on three *Hylocereus* species. (A) Effect of rooting agents on shoot emission time (SET); (B) effect of substrates on shoot emission time (SET); (C) effect of rooting agents on cutting viability (CV); and (D) effect of substrates on cutting viability (CV). Different letters above the bars indicate statistically significant differences according to Tukey's test ($p \leq 0.05$).

The species factor (C) had a significant effect on shoot emission time (SET) and cutting viability (CV) ($p < 0.05$) (Supplementary Table S1). Tukey's multiple comparison test identified three statistically distinct groups for both variables. For SET, *H. costaricensis* and *H. undatus* exhibited the shortest emission times, whereas *H. megalanthus* showed the longest SET values (Figure 4A). With respect to CV, *H. costaricensis* displayed the highest viability, *H. megalanthus* the lowest, and *H. undatus* intermediate values (Figure 4B).

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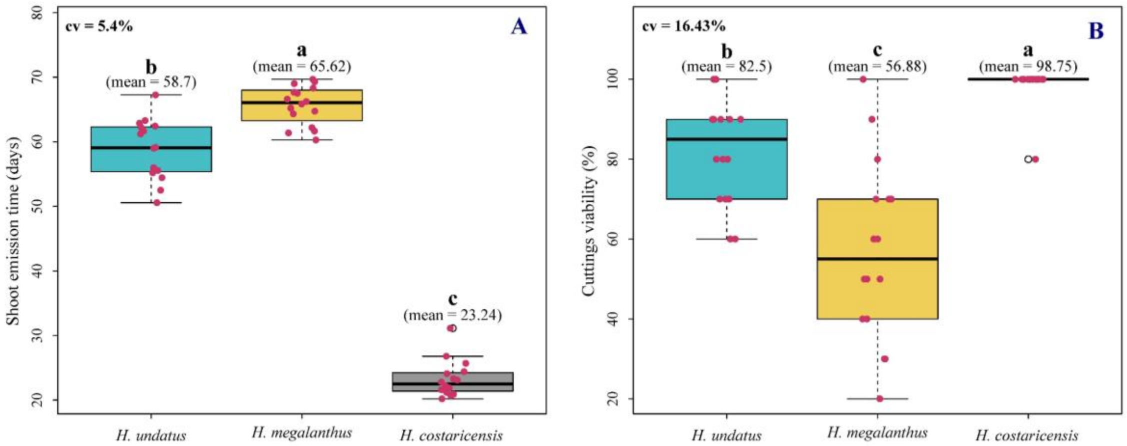

Figure 4. Performance of three *Hylocereus* species in establishment variables. (A) Shoot emission time (SET) and (B) cutting viability (CV). Different letters above the bars indicate statistically significant differences according to Tukey's test ($p \leq 0.05$).

3.2. *Vegetative development*

The variables number of shoots (NS), shoot length (SL), number of roots (NR), and root length (RL) were used to assess the vegetative development of pitahaya seedlings. Figure 5 shows representative plants from the 12 treatments evaluated, where marked morphological differences were observed in both the aerial and root systems. Treatment T10 displayed well-developed aerial and root structures compared to the other treatments.

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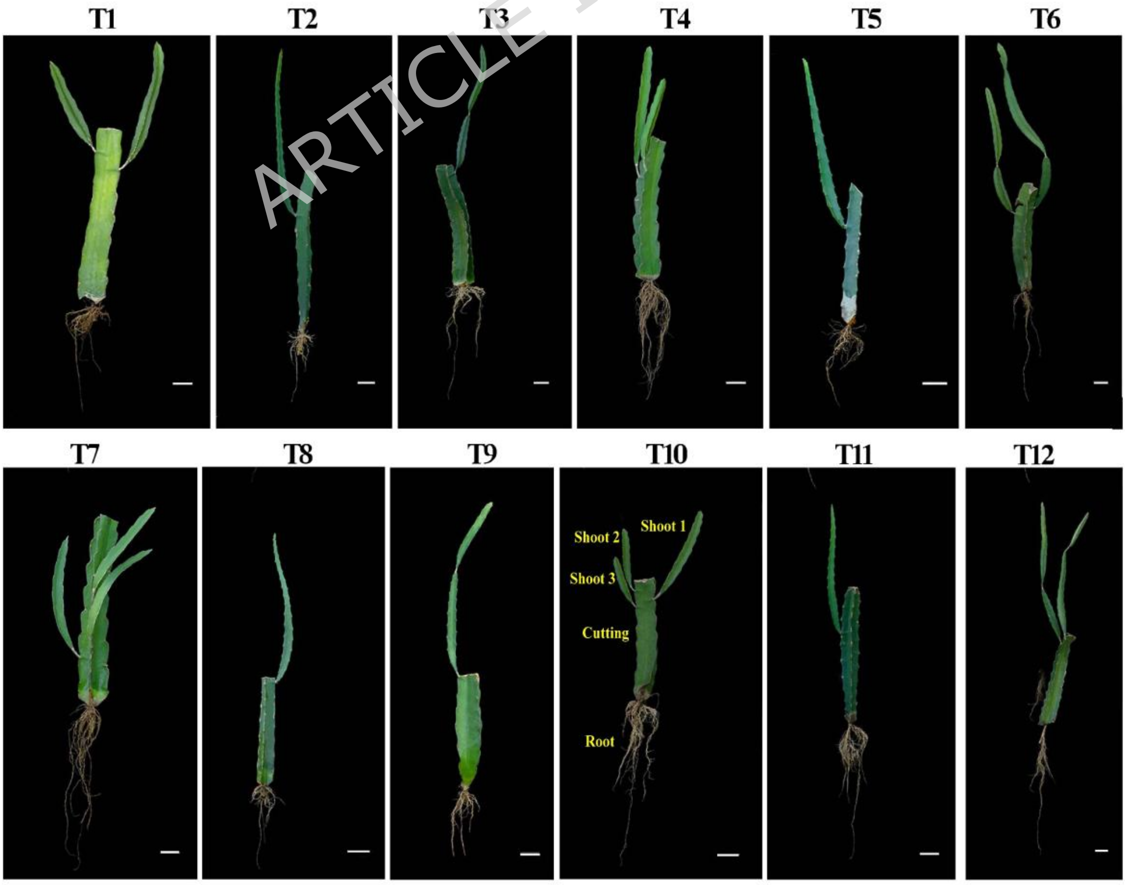

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Figure 5. Pitahaya seedlings corresponding to the 12 treatments evaluated in the study. Scale bar = 5 cm.

The ANOVA revealed significant differences among treatments (A × B × C) for number of shoots (NS), shoot length (SL), and root length (RL) ($p < 0.05$), whereas no significant effect was observed for number of roots (NR). The coefficients of variation for these variables ranged from 8.46% to 13.12% (Supplementary Table S3).

Figure 6A shows the number of shoots (NS), where Tukey's multiple comparison test identified six statistically distinct groups. Treatment T7 presented the highest mean (1.62 shoots per plant; group a). In contrast, T2 and T8 exhibited the lowest values, with means of 1.06 and 1.08 shoots per plant, respectively (groups d and cd). The remaining treatments exhibited intermediate values, forming groups ab, abc, and bcd.

For shoot length (SL) (Figure 6B), Tukey's test also separated treatments into six statistically distinct groups. Treatments T3, T9, T12, and T6 showed the greatest mean shoot lengths (68.67, 67.99, 60.94, and 57.83 cm per plant, respectively; group a). Conversely, T5 and T2 had the lowest mean SL values (23.30 and 24.07 cm per plant, respectively; group d), whereas treatments classified into groups b, bc, bcd, and cd exhibited intermediate shoot lengths.

Figure 6C shows the number of roots (NR). Although the overall ANOVA did not detect significant differences among treatments, Tukey's multiple comparison test distinguished five statistically homogeneous groups. The highest mean NR values were observed in treatments T3 and T12 (5.30 and 5.23 roots per plant, respectively; group a). In contrast, T8, T2, T11, and T5 exhibited the lowest means (3.28, 3.34, 3.43, and 3.49 roots per plant, respectively; group c). The remaining treatments displayed intermediate NR values (groups ab, abc, and bc).

For root length (RL) (Figure 6D), Tukey's test identified ten statistically distinct groups. Treatments T12 and T10 presented the greatest mean RL values (42.69 and 41.38 cm per plant, respectively; groups a and ab), whereas T8 and T2 showed the shortest root lengths (14.35 and 14.74 cm per plant, respectively; group g). The remaining treatments exhibited intermediate RL values, classified into groups abc, abcd, bcd, cd, de, ef, and fg.

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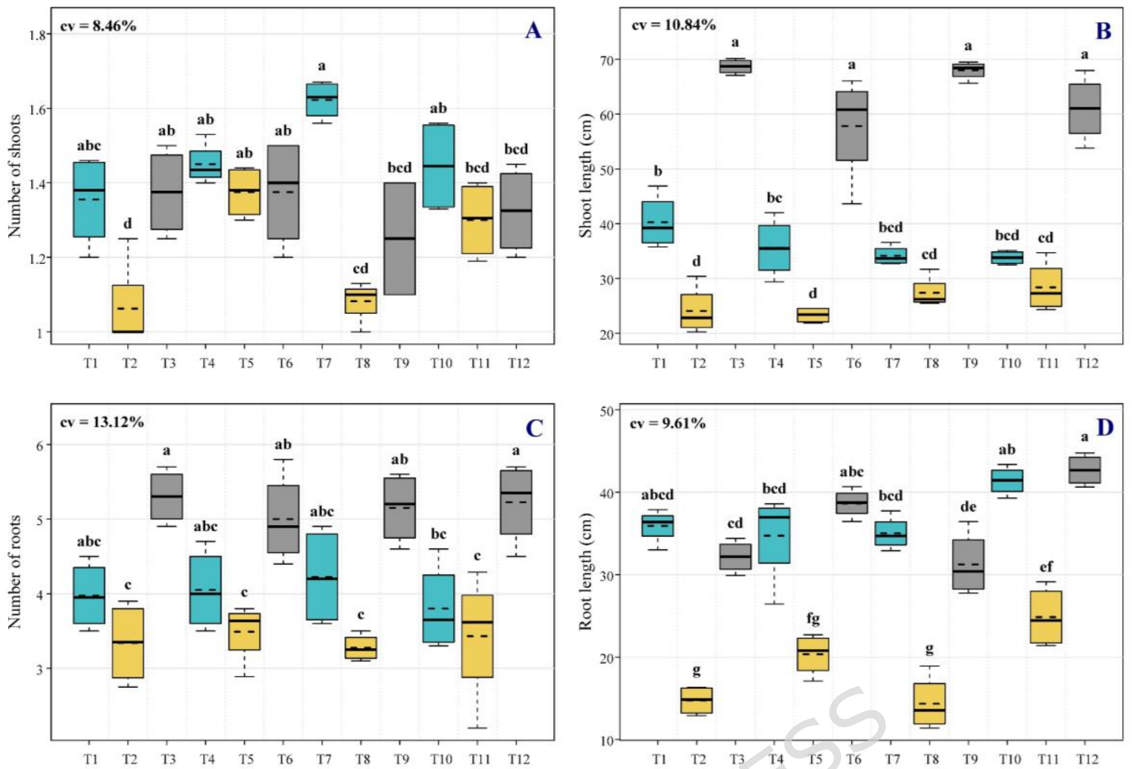

Figure 6. Effects of 12 treatments (A × B × C) on growth variables of pitahaya seedlings. (A) Number of shoots (NS); (B) shoot length (SL); (C) number of roots (NR); and (D) root length (RL). Different letters above the bars indicate statistically significant differences according to Tukey's test ($p \leq 0.05$).

Figure 7 shows the response of the three *Hylocereus* species in terms of vegetative growth variables. ANOVA indicated significant differences among species for number of shoots (NS) and shoot length (SL) ($p < 0.05$), with coefficients of variation of 8.46% and 10.48%, respectively (Supplementary Table S3). For NS (Figure 7A), Tukey's multiple comparison test identified three statistically distinct groups. *H. undatus* and *H. costaricensis* produced the highest numbers of shoots (1.47 and 1.33 shoots per plant; groups a and b, respectively), whereas *H. megalanthus* exhibited the lowest mean value (1.21 shoots per plant; group c). For SL (Figure 7B), Tukey's test also distinguished three statistically distinct groups. *H. costaricensis* presented the greatest mean shoot length (63.83 cm; group a), while *H. megalanthus* showed the lowest value (25.79 cm; group c). *H. undatus* displayed an intermediate mean value and was classified in group b.

Figures 7C and 7D show the response of the pitahaya species for number of roots (NR) and root length (RL), respectively. ANOVA indicated highly significant differences among species for both variables ($p < 0.001$), with coefficients of variation of 13.12% for NR and 9.61% for RL (Supplementary Table S3). For NR (Figure 7C), Tukey's multiple comparison test identified three statistically distinct groups. *H. costaricensis* presented the highest mean number of roots (5.17 roots per plant; group a), whereas *H. megalanthus* exhibited the lowest value (3.38 roots per plant; group c). *H. undatus* showed an intermediate mean and was classified in group b. For RL (Figure 7D), Tukey's test distinguished two statistically distinct groups. *H. costaricensis* and *H. undatus* exhibited

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the greatest mean root lengths (36.19 and 36.76 cm, respectively; group a), while *H. megalanthus* recorded the lowest mean value (18.57 cm; group b).

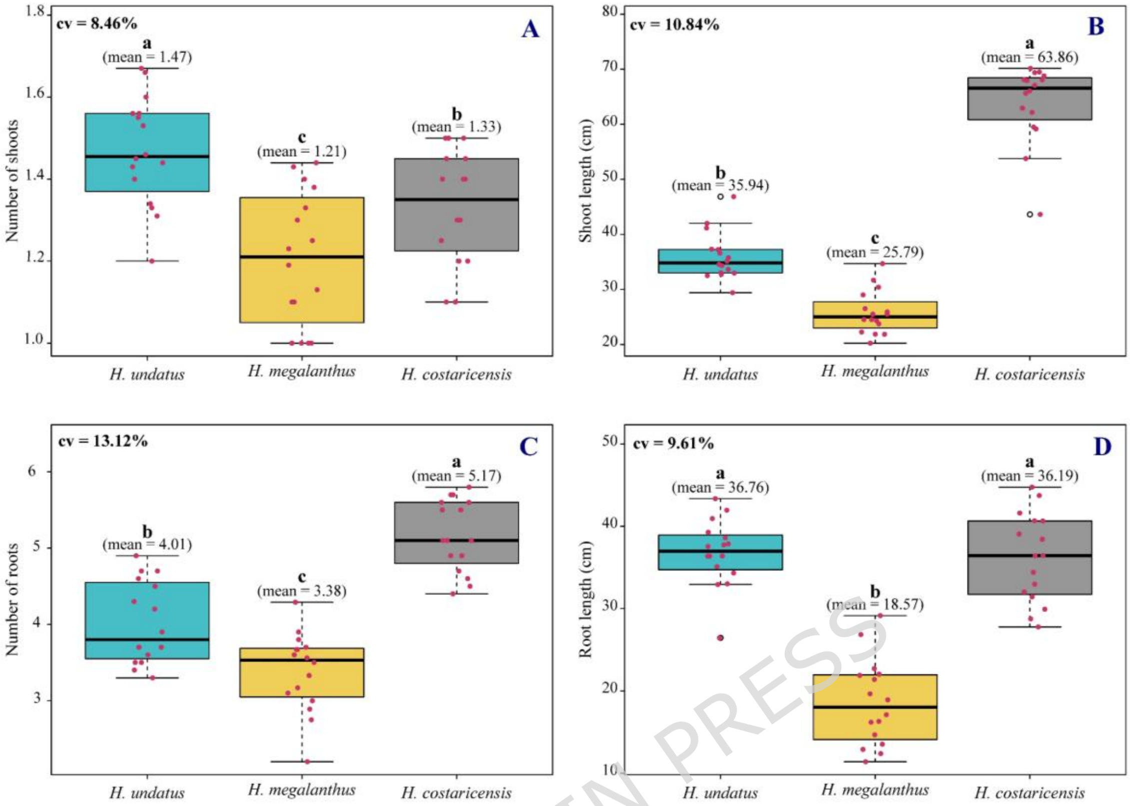

Figure 7. Growth response of three pitahaya species. A) Number of shoots, B) Shoot length, C) Number of roots, and D) Root length. Different letters indicate statistically different groups according to the Tukey test ($p \leq 0.05$).

3.3. Vegetative vigor index (VVI)

Figure 8A shows the mean values of the 12 treatments (A × B × C) for the Vegetative Vigor Index (VVI). The ANOVA revealed significant differences ($p < 0.05$) with a coefficient of variation of 15.61% (Supplementary Table S4). Tukey's multiple comparison test identified five statistically distinct groups. Treatments T12, T3, T9, and T6 presented the highest VVI values (110.18, 107.52, 105.64, and 98.41, respectively; group a), whereas T2 and T8 exhibited the lowest means (18.19 and 18.20, respectively; group d). The remaining treatments, classified into groups b, bc, and cd, displayed intermediate VVI values (Figure 8A).

The VVI also differed significantly among *Hylocereus* species ($p < 0.0001$). According to Tukey's test, *H. costaricensis* showed the highest mean index value (105.44; group a), while *H. megalanthus* exhibited the lowest mean (28.56; group c). *H. undatus* displayed an intermediate VVI and was classified in group b (Figure 8B).

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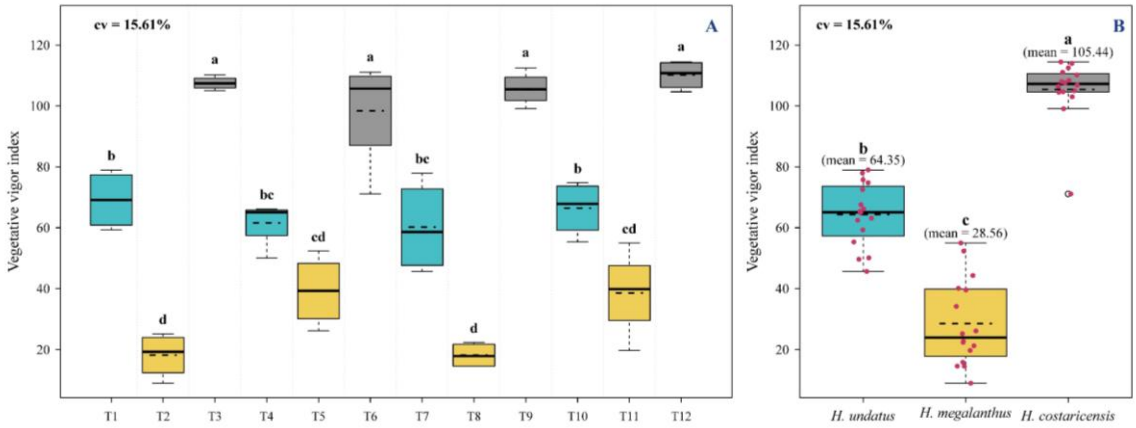

Figure 8. Vegetative vigor index (VVI) of (A) 12 treatments and (B) three *Hylocereus* species. Different letters above the boxes indicate statistically significant differences according to Tukey's test ($p \leq 0.05$).

Effect size estimates (ηp^2) and binomial GLM results consistently identified species as the most influential factor affecting propagation performance. Species explained between 55% and 98% of the observed variance in growth-related traits and showed the strongest effect on cutting viability, whereas rooting agents and substrates generally contributed less to the observed variation (Supplementary Tables S1–S4).

3.4. Spearman correlation between evaluated variables

Spearman's correlation analysis (Figure 9) showed significant associations among most of the variables evaluated. Due to its inverse nature, shoot emission time (SET) exhibited negative correlations with all other variables: shoot length (SL) ($\rho = -0.90$*), number of roots (NR) ($\rho = -0.77$*), root length (RL) ($\rho = -0.51$*), cutting viability (CV) ($\rho = -0.73$*), and vegetative vigor index (VVI) ($\rho = -0.85$*). In contrast, number of shoots (NS) showed weak correlations with most variables ($\rho \approx 0.16$–0.25), although a moderate positive correlation was observed with RL ($\rho = 0.49$*).

Among the vegetative growth variables, strong positive correlations were detected between SL and NR ($\rho = 0.73$*), RL ($\rho = 0.57$*), CV ($\rho = 0.76$*), and VVI ($\rho = 0.89$*). Likewise, NR was positively correlated with CV ($\rho = 0.81$*), VVI ($\rho = 0.80$*), and RL ($\rho = 0.46$*). The strongest positive association was observed between CV and VVI ($\rho = 0.92$*).

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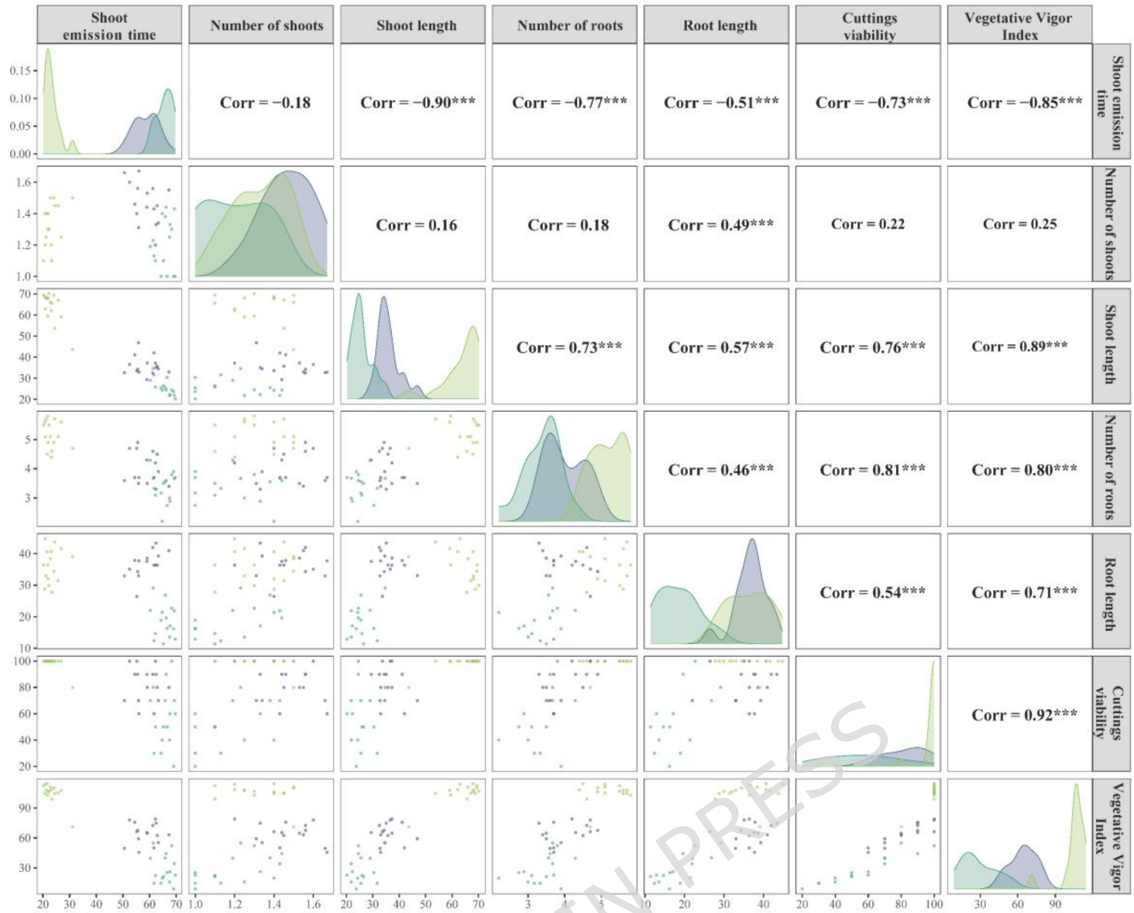

Figure 9. Spearman correlation matrix with density and scatter plots by species for the seven variables evaluated in *Hylocereus* spp.

3.5. Principal component analysis (PCA) of the variables evaluated

The principal component analysis (PCA) explained 87.3% of the total variance based on the first two dimensions (Dim1 = 69.3% and Dim2 = 18.0%) for the three *Hylocereus* species (Figure 10). Dim1 represented a vegetative performance gradient, with strong positive loadings for shoot length (SL), number of roots (NR), root length (RL), cutting viability (CV), and vegetative vigor index (VVI), and a negative loading for shoot emission time (SET), reflecting its inverse relationship with the other traits. Thus, higher Dim1 values indicated more vigorous seedlings, characterized by greater rooting capacity and earlier shoot emergence. Dim2 captured secondary variation primarily associated with the number of shoots (NS).

The species exhibited distinct distributions along the PCA axes. *H. costaricensis* was positioned toward higher values of SL, NR, RL, CV, and VVI, whereas *H. megalanthus* was associated with higher SET values. *H. undatus* occupied an intermediate position and was associated with higher NS values.

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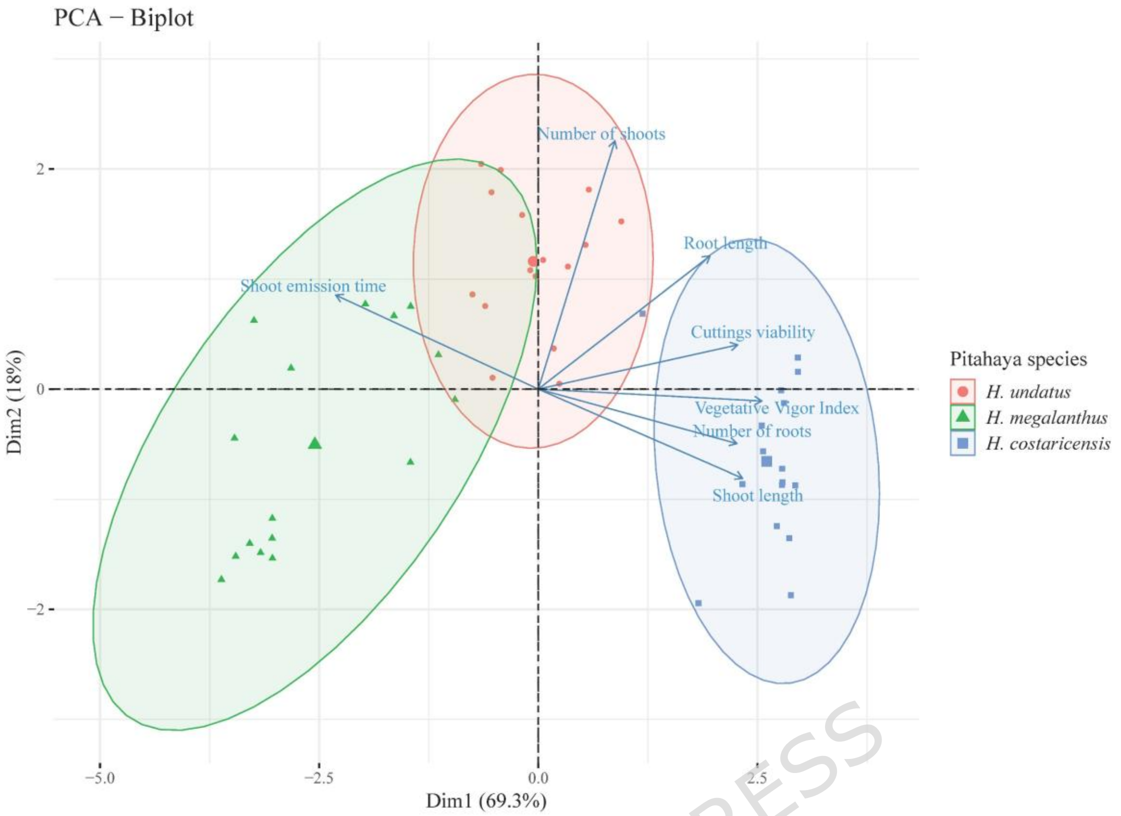

Figure 10. PCA biplot of vegetative growth variables in *Hylocereus* species, showing the first two principal components (Dim1 and Dim2), variable loading vectors, and 95% confidence ellipses for species grouping.

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4. Discussion

The results indicate that vegetative propagation of pitahaya through cuttings is strongly influenced by rooting agents, substrate type, species-specific responses, and the interaction among these factors. Regarding shoot emission time (SET), *H. costaricensis* consistently exhibited the shortest emission times, indicating faster shoot emergence compared with the other species evaluated. These findings agree with those reported by Chhetri et al.[8], who observed earlier sprouting in *H. costaricensis* cuttings relative to other pitahaya varieties.

In contrast, Anagha et al.[38] reported a mean sprouting time of 13.2 days for *H. undatus* grown in vermicompost, which is considerably shorter than the minimum value recorded in the present study (21.53 days). This discrepancy may be associated with substrate composition, as vermicompost is rich in readily available nutrients and supports high microbial activity, particularly enhancing nitrogen (N) and phosphorus (P) availability [39–41]. These conditions can accelerate metabolic processes related to bud activation and shoot emergence, thereby reducing the time required for sprouting.

The faster shoot emergence observed in *H. costaricensis* may also be associated with differences in endogenous hormonal balance and reserve mobilization. Adventitious shoot formation depends largely on the interaction between auxins, cytokinins, and carbohydrate availability. Species with a more favorable hormonal balance can activate dormant meristems more rapidly, resulting in earlier sprouting and improved

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establishment. In addition, greater mobilization of stored reserves may support the metabolic demands required for bud activation and initial shoot development.

Similarly, Cardoso et al.[42], who evaluated five *Hylocereus* species, reported that *H. undatus* exhibited greater rooting capacity and higher cutting viability than *H. costaricensis*. This contrasts with the findings of the present study, in which *H. costaricensis* showed the highest cutting viability (CV), surpassing *H. undatus*. In addition, *H. megalanthus* consistently exhibited the lowest CV values regardless of the rooting agent or substrate used. The reduced viability observed in this species may be associated with its ecological requirements, as *H. megalanthus* is better adapted to altitudinal ranges between 800 and 1800 m a.s.l. [43]. Differences in environmental adaptation may therefore influence rooting responses and propagation success among species.

Regarding the variables associated with vegetative development, the responses of number of shoots (NS) and shoot length (SL) varied among treatments. At the interaction level (A × B × C), the highest NS value was observed in *H. undatus* established in substrate 2 and treated with NAA + IBA (T7), reaching a mean of 1.6 shoots per plant (Figure 5A). In comparison, Filho et al.[44] reported an average of 3.25 shoots per plant in *H. undatus* cuttings treated with IBA, a value considerably higher than that obtained in the present study. This difference, despite involving the same species and a similar rooting inducer, may be attributed to variation in substrate composition and environmental conditions, which play a key role in vegetative propagation processes [13].
For shoot length (SL), the highest mean value was recorded in *H. costaricensis* under the combination of substrate 1 and seaweed extract (T3), reaching 68.67 cm after four months of evaluation. By comparison, Cardoso et al.[42] reported shoot lengths of 27.25 cm and 22.18 cm for *H. undatus* and *H. costaricensis*, respectively, at 82 days, while Chhetri et al.[8] observed 52.1 cm at 90 days. These differences are likely associated with variation in evaluation period, as longer experimental durations generally allow greater shoot elongation to be expressed. Supporting this interpretation, Navarrete Torres et al.[45] reported a total plant height of 263.40 cm in *H. undatus* after nine months, highlighting the strong influence of developmental time on shoot growth in pitahaya.

Successful rooting during vegetative propagation is strongly influenced by substrate moisture conditions and the use of rooting stimulants [46,47]. In the present study, the number of roots (NR) variable showed that treatments T3 and T12 produced the highest averages, with 5.30 and 5.23 roots per plant, respectively. This indicates that the species *H. costaricensis* and substrate 1 were the most decisive factors, while the type of rooting agent had no significant influence.
By comparison, Cardoso et al.[42] evaluated the effect of biostimulants on pitahaya cuttings and reported averages of 15.13 and 9.13 roots per plant for *H. undatus* and *H. costaricensis*, respectively, using a commercial substrate enriched with river sand. These differences could be attributed to the physical and chemical properties of the substrate, as such formulations are specifically designed to optimize moisture balance and aeration for vegetative propagation.

With respect to root length (RL), the highest values were observed in treatments combining NAA + IBA and substrate 1, particularly in *H. costaricensis* and *H. undatus*

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(treatments T12 and T10), which reached mean root lengths of 42.69 cm and 41.38 cm, respectively, after four months of evaluation. Previous studies have reported lower RL values, including 24.07 and 19.00 cm in *H. undatus* and *H. costaricensis* at 82 days [42], 25.2 cm in *H. costaricensis* at 90 days [8], and 13.6 cm in *H. megalanthus* at 90 days [48]. These differences are likely associated with variation in evaluation period, as the cited studies assessed rooting for less than four months.
However, Navarrete Torres et al.[45] reported a maximum root length of 22.89 cm in *H. undatus* after nine months, suggesting that although evaluation time can influence root development, root elongation dynamics may vary depending on species and propagation conditions, particularly during early stages of vegetative establishment. Taken together, these results indicate that root development is influenced by both species-specific responses and the physicochemical properties of the substrate.

The vegetative vigor index (VVI) integrates all evaluated variables except shoot emission time (SET), as this variable is inversely related to the other growth parameters. The results showed that treatments involving *H. costaricensis* consistently exhibited the highest VVI values. In particular, treatment T12 recorded the highest index, indicating that the interaction between *H. costaricensis*, NAA + IBA, and substrate 1 was associated with the greatest vegetative vigor.
Notably, both the highest and lowest VVI values were obtained with substrate 1, suggesting that substrate alone may not fully determine variation in this index. Nevertheless, previous studies emphasize that substrate characteristics play a critical role in the vegetative propagation of pitahaya [49]. According to Campbell et al.[50], selecting an appropriate substrate is essential, as low porosity can promote waterlogging and reduce oxygen availability in the rhizosphere [13]. In this study, substrate 1 may have contributed to improved vegetative development due to its composition. The combination of forest soil, compost, and cattle manure likely enhances nutrient availability, organic matter content, and microbial activity, which can promote root formation and overall plant growth [13,51].

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Not all plants respond uniformly to a specific type of stimulant; this response may vary even among individuals of the same species [52]. In the present study, rooting agents significantly influenced the vegetative vigor index (VVI), with the combination of NAA + IBA being associated with higher VVI values compared to the seaweed extract.
However, several studies have reported beneficial effects of seaweed-based products on vegetative propagation, attributing their activity to phenolic compounds and hormone-like substances that enhance endogenous auxin activity and nutrient assimilation [53–56]. Indeed, [57] observed superior growth responses with seaweed extracts compared to synthetic NAA in lettuce and tomato transplants, reflected in increased biomass accumulation and dry matter content. These contrasting findings suggest that the effectiveness of biostimulants may depend on species-specific responses, physiological status of the cuttings, and environmental conditions during propagation.

Auxin based rooting agents containing NAA and IBA, which were associated with higher effectiveness than seaweed extract under the conditions of this study, are among the most widely used compounds in vegetative propagation due to their consistent efficacy across a wide range of plant species [17]. Their activity is primarily related to the stimulation of cell division, elongation, and differentiation processes that are essential for adventitious

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root initiation and development [58,59]. Numerous studies have reported that IBA, in particular, tends to promote superior rooting responses in cuttings compared with other plant growth regulators [60–62].

The superior performance observed in treatments receiving NAA + IBA may be explained by the complementary physiological roles of these auxins during adventitious root formation. Auxins stimulate cell dedifferentiation, cell division, and the development of root primordia, while also enhancing vascular reconnection between the cutting and newly formed roots. Root formation increases water and nutrient uptake capacity, which subsequently supports shoot elongation and overall vegetative growth. These physiological processes may explain the positive responses observed for shoot length, root length, and vegetative vigor.

The contrasting physicochemical properties of the substrates may have contributed to the observed responses. Substrate 1 exhibited higher organic matter, total nitrogen, and electrical conductivity than Substrate 2 (Supplementary Table S5), potentially enhancing nutrient availability and root establishment. In addition, the effectiveness of exogenous auxins is influenced by substrate conditions, as adequate moisture and aeration favor root primordia formation and root elongation. Therefore, the superior performance observed in several treatments likely resulted from the interaction between hormonal stimulation and substrate properties.Although species of the genus *Hylocereus* can be cultivated under marginal agronomic conditions and warm climates [63,64], their physiological responses can vary and may be constrained by thermal conditions, which are a determining factor for plant growth and development [65]. Based on the morphological differences observed across treatments (Figure 6), the ANOVA results, and the effect size analyses, species identity emerged as the most influential factor affecting vegetative propagation in pitahaya. Effect size analysis (ηp^2) for growth-related traits and binomial GLM results for cutting viability consistently identified species as the most influential factor affecting propagation performance (Supplementary Tables S1–S4). The dominant influence of species likely reflects intrinsic ecophysiological differences, including growth dynamics, hormonal balance, and rooting capacity, which determine propagation performance under nursery conditions.

Among the 12 treatments evaluated, *H. costaricensis* consistently exhibited shorter shoot emission time (SET), higher cutting viability (CV), greater shoot length (SL), number of roots (NR), and root length (RL), whereas number of shoots (NS) did not follow this same pattern. In contrast, *H. megalanthus* generally showed the lowest values for most vegetative growth variables, while *H. undatus* displayed intermediate responses. These differences suggest the presence of species-specific morphological and physiological traits that influence vegetative propagation responses.

In particular, *H. megalanthus* appears to be less adapted to hot and arid environments compared with *H. costaricensis* and *H. undatus* [66,67]. which may partly explain its reduced rooting and growth responses under the conditions of this study. Furthermore, it has been demonstrated that plant responses to hormonal stimuli depend on the biochemical, physiological, and molecular capacities of each tissue to perceive and transduce these signals [68,69], highlighting the importance of species-specific sensitivity to growth regulators during vegetative propagation.

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These findings indicate that vegetative propagation of pitahaya is governed by a complex interaction among species-specific genetic, physiological, and environmental factors. Although rooting agents and substrate type influenced vegetative development, their effects were strongly modulated by species identity and prevailing propagation conditions. The results provide robust evidence for optimizing propagation protocols in *Hylocereus* spp. and underscore the need for further research addressing field adaptation, as well as the physiological and molecular mechanisms underlying the interspecific differences observed. Collectively, these insights contribute to improving propagation efficiency, strengthening production strategies, and promoting the long-term sustainability of pitahaya cultivation in tropical regions.

Some limitations of this study should be acknowledged. The experiment was conducted under nursery conditions over a relatively short period and without the same level of environmental control as growth chamber studies. Furthermore, although the cuttings were obtained from healthy and uniform mother plants, some degree of biological variability may have influenced the observed responses. In addition, the study focused on morphological and vegetative growth traits without addressing the physiological or molecular mechanisms underlying rooting and shoot development. Therefore, future research should evaluate the field performance of propagated plants and investigate the physiological, biochemical, and molecular processes that regulate rooting, vegetative growth, and species-specific responses.

5. Conclusions

Vegetative propagation of pitahaya was influenced by the interaction among multiple factors, with species identity emerging as the primary determining factor. Effect size analysis (ηp^2) confirmed that species explained the largest proportion of variance across all evaluated variables. Among the species evaluated, *Hylocereus costaricensis* exhibited the most consistent and favorable responses in establishment and vegetative growth variables, as well as the highest vegetative vigor index (VVI).

Regarding rooting agents, the application of NAA + IBA was associated with higher responses than the seaweed extract (*Ecklonia maxima*), particularly in root development and overall seedling vigor, supporting the effectiveness of synthetic auxins in stimulating cell division and elongation processes. With respect to the propagation medium, substrate 1 composed of forest soil, compost, and cattle manure resulted in higher values for root and vegetative growth variables compared with the other substrates evaluated.

The interaction between *H. costaricensis*, substrate 1, and NAA + IBA (treatment T12) produced the highest VVI, identifying this combination as the most favorable for vegetative propagation under nursery conditions. Collectively, these findings provide a scientific basis for improving pitahaya propagation protocols under nursery conditions. However, further field validation is required to confirm seedling performance after transplantation and to support large-scale implementation.

CRediT authorship contribution statement

B.C.C: Writing – original draft, Formal Analysis, Data Curation, Conceptualization; **J.M.C.M:** Research, Methodology; **J.A.C.S:** Data Curation, Formal Analysis; **M.A.I:** Research, Methodology; **V.H.T.M:** Project Management, Financial Procurement;

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D.G.F: Visualization, Formal Analysis; **J.T.C:** Investigation, Methodology; **M.G:** Visualization, Validation; **D.T:** Visualization, Validation, Formal Analysis.

Data availability
Data will be made available on request.

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Declaration of competing interest
The authors declare that they have no conflicts of interest. The authors declare that they have no competing financial interests or personal relationships that could influence the work presented in this article.

Supplementary material
Supplementary material associated with this article can be found, in the online version, at

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6. Bibliography

1. Kishore, K. Phenological growth stages of dragon fruit (*Hylocereus undatus*) according to the extended BBCH-scale. *Sci. Hortic.* **213**, 294–302 (2016).

2. Jakobina, M., Łyczko, J., Zydorowicz, K., Galek, R. & Szumny, A. The Potential Use of Plant Growth Regulators for Modification of the Industrially Valuable Volatile Compounds Synthesis in *Hylocreus undatus* Stems. *Molecules* **28**, 3843 (2023).

3. Mercado-Silva, E. M. Pitaya—*Hylocereus undatus* (Haw). *Exotic Fruits Reference Guide* 339–349 (2018) doi:10.1016/B978-0-12-803138-4.00045-9.

4. Verona-Ruiz, A., Urcia-Cerna, J. & Paucar-Menacho, L. M. Pitahaya (*Hylocereus* spp.): Cultivo, características fisicoquímicas, composición nutricional y compuestos bioactivos. *Scientia Agropecuaria* **11**, 439–453 (2020).

5. Carmen, F., Frances, C. & Barthe, L. Trends on valorization of pitaya fruit biomass through value-added and green extraction technology – A critical review of advancements and processes. *Trends Food Sci. Technol.* **138**, 339–354 (2023).

6. Ministerio de Desarrollo Agrario y Riego (MIDAGRI). *Dinámica de La Producción y Precios de La Pitahaya En El Perú, 2019 - 2024 (a Julio)*. https://cdn.www.gob.pe/uploads/document/file/2055424/Perfil%20de%20Mercado%20de%20la%20Pitahaya.pdf.pdf (2024).

7. Singh, R. P., Prasad, P. V. V. & Reddy, K. R. Climate Change: Implications for Stakeholders in Genetic Resources and Seed Sector. in *Advances in Agronomy* vol. 129 117–180 (Academic Press, 2015).

ARTICLE IN PRESS

8. Chhetri, S., Alam, M., Hasan, M. A. & Lama, A. The efficacy of varying length of cladode cutting and IBA concentration on root and shoot growth in dragon fruit (*Hylocereus costaricensis* [F.A.C. Weber] Britton and Rose.) cv. Royal Moroccan Red). *Journal of Crop and Weed* **18**, 190–194 (2022).

9. Navarro-Sandoval, B. G. & Canales-Carrera, E. E. Efecto de diferentes concentraciones de benzilaminopurina (BAP) sobre el establecimiento in vitro de pitahaya (*Hylocereus guatemalensis*). *Revista de Innovación y Transferencia Productiva* **2**, (2021).

10. López-Medina, S. E. *et al.* Efecto del ácido indolbutírico en la propagación de *Hylocereus undatus* "pitahaya" bajo condiciones de invernadero. *Manglar* **20**, 341–346 (2023).

11. Solano Vargas, J. J., Sanchez-Santillan, T., Arévalo López, L. A. & Morales Rojas, E. Efecto de sustratos y enraizadores en la propagación vegetativa de *Coffea arabica* var. típica en microtúneles. *Ciencia y Tecnología Agropecuaria* **24**, 2627 (2023).

12. de Sousa Antunes, L. F. *et al.* Millicompost: Sustainable substrate for the production of dragon fruit seedlings (*Selenicereus undatus*). *Clean. Eng. Technol.* **4**, 100107 (2021).

13. Nkongho, R. N. *et al.* Vegetative propagation of F1 tomato hybrid (*Solanum lycopersicum* L.) using different rooting media and stem-nodal cuttings. *J. Agric. Food Res.* **11**, 100470 (2023).

14. Wu, Y. *et al.* Continuous light induces adventitious root formation in *Robinia pseudoacacia* L. through the auxin response factor RpARF12 lacking the CTD domain. *Plant Physiology and Biochemistry* **226**, 110049 (2025).

15. Juárez-Rosete, C. R. *et al.* Medios de enraizamiento y aplicación de auxinas en la producción de plántulas de fresa. *Ciencia y Tecnología Agropecuaria* **21**, 71–83 (2020).

16. Vergara Garces, R. & Caycho Medrano, N. Efecto de tres dosis de bioestimulante con dos tipos de sustrato en la propagación asexual de estacas de pitahaya roja (*Hylocereus hybridum*), bajo condiciones de invernadero, Independencia – Huaraz - Ancash. *Aporte Santiaguino* **17**, (2024).

17. Mendoza, W. *et al.* Determinación de las concentraciones adecuadas de 2,4 diclorofenoxiacético y Kelpak en el enraizamiento de estacas de *Vaccinium floribundum* Kunth "pushgay". *Manglar: Revista de Investigación Científica, ISSN-e 2414-1046, ISSN 1816-7667,* **17**, 21–25 (2020).

18. Craigie, J. S. Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol.* **23**, 371–393 (2011).

19. Featonby-Smith, B. C. & van Staden, J. The effect of seaweed concentrate and fertilizer on growth and the endogenous cytokinin content of Phaseolus vulgaris. *South African Journal of Botany* **3**, 375–379 (1984).

ARTICLE IN PRESS

ARTICLE IN PRESS

20. Crouch, I. J. & van Staden, J. Evidence for the presence of plant growth regulators in commercial seaweed products. *Plant Growth Regul.* **13**, 21–29 (1993).

21. Pienaar, B. C. *et al.* Mitigating salt stress in maize using *Ecklonia maxima* seaweed extracts. *Plant Stress* **16**, 100828 (2025).

22. Tariq, T. B., Karishma, Umer, M. & Mubeen-ur-Rehman. The potential of seaweed-derived polysaccharides as sustainable biostimulants in agriculture. *Int. J. Biol. Macromol.* **298**, 140009 (2025).

23. Zheng, T. *et al.* Optimal rooting substrates and hormonal regulation via a multi-omics analysis during Vitis davidii cutting rooting. *Plant Stress* **16**, 100851 (2025).

24. Fernández-Suárez, K., Gil-Pérez, M., Medina-García, L. R. & Hasaert, G. Beneficios de la cáscara de arroz como cobertura en una plantación de aguacate (*Persea americana* L.). *Cultivos Tropicales* **43**, e12 (2023).

25. Howe, J. A. *et al.* Influence of fertilizer and manure inputs on soil health: A review. *Soil Security* **16**, 100155 (2024).

26. Servicio Nacional de Meteorología e Hidrología del Perú (SENAMHI). Mapa Climático del Perú. https://www.senamhi.gob.pe/?p=mapa-climatico-del-peru (2020).

27. Nico, A. I., Jiménez-Díaz, R. M. & Castillo, P. Solarization of soil in piles for the control of Meloidogyne incognita in olive nurseries in southern Spain. *Plant Pathol.* **52**, 770–778 (2003).

28. Cruz Rosero, N., Morante Carriel, J. & Acosta Anzules, M. Propagación vegetativa de Fernansánchez (*Triplaris guayaquilensis*) mediante la utilización de hormonas de enraizamiento (ANA y AIB). https://revistas.uteq.edu.ec/index.php/cyt/article/view/68/82 (2008).

29. Espinosa-Antón, A. A., Hernández-Herrera, R. M. & González González, M. Extractos bioactivos de algas marinas como bioestimulantes del crecimiento y la protección de las plantas. *Artículo de revisión Biotecnología Vegetal* **20**, 257–282 (2020).

30. Jose, S. & Sivaprasad, A. Influence of gibberellic acid (GA3) on in vitro seed germination of *Dendrocalamus strictus* (Roxb.) Nees: Exploring its effect under varied storage periods and micropropagation potential. *Advances in Bamboo Science* **11**, 100141 (2025).

31. BharathKumar, S. *et al.* Unveiling the influence of NPK, organic fertilizers, and plant growth enhancers on China aster (*Callistephus chinensis* L.) CV. ‘Arka Kamini’ seed yield. *Ind. Crops Prod.* **227**, 120778 (2025).

32. Fetouh, M. I. & Hassan, F. A. Seed germination criteria and seedling characteristics of *Magnolia grandiflora* L. trees after cold stratification treatments. *Int.J.Curr.Microbiol.App.Sci* **3**, 235–241 (2014).

33. R Core Team. A language and environment for statistical computing. Preprint at https://www.R-project.org/ (2025).

ARTICLE IN PRESS

ARTICLE IN PRESS

34. Wickham, H., François, R., Henry, L. & Müller, K. dplyr: A Grammar of Data Manipulation. Preprint at https://CRAN.R-project.org/package=dplyr (2023).

35. de Mendiburu, F. agricolae: Statistical Procedures for Agricultural Research. Preprint at https://CRAN.R-project.org/package=agricolae (2023).

36. H. Wickham. *Ggplot2: Elegant Graphics for Data Analysis*. (2016).

37. Wei, T. & Simko, V. R package 'corrplot': Visualization of a Correlation Matrix. Preprint at https://github.com/taiyun/corrplot (2021).

38. Anagha, P. K. *et al.* Enhancing Dragon Fruit (*Hylocereus undatus*) Propagation Efficiency through Growing Media. *International Journal of Economic Plants* **12**, 1–5 (2025).

39. Hussain, N. *et al.* Excellent N-fixing and P-solubilizing traits in earthworm gut-isolated bacteria: A vermicompost based assessment with vegetable market waste and rice straw feed mixtures. *Bioresour. Technol.* **222**, 165–174 (2016).

40. Bouchtaoui, E. M. *et al.* Harnessing compost and vermicompost for sustainable and effective management of plant-parasitic nematodes in agricultural systems: A critical review. *Physiol. Mol. Plant Pathol.* **133**, 102363 (2024).

41. Tóthné Bogdányi, F. *et al.* Composted Municipal Green Waste Infused with Biocontrol Agents to Control Plant Parasitic Nematodes—A Review. *Microorganisms 2021, Vol. 9, Page 2130* **9**, 2130 (2021).

42. Felipe Cardoso, J. *et al.* Effect of different doses of biostimulate on the rooting and development of pitaya genotypes cuttings. *Revista de Ciencias Agroveterinarias* **23**, 604–611 (2024).

43. Tuesta-Trauco, K. M. *et al.* Land Suitability for Pitahaya (*Hylocereus megalanthus*) Cultivation in Amazonas, Perú: Integrated Use of GIS, RS, F-AHP, and PROMETHEE. *Remote Sensing 2025, Vol. 17, Page 637* **17**, 637 (2025).

44. Filho, F. S. T. P., Almeida, E. I. B., Barroso, M. M. A., Cajazeira, J. P. & de Medeiros Corrêa, M. C. Comprimento de estacas e concentrações de ácido indolbutírico (AIB) na propagação vegetativa de pitaia. *Revista Ciência Agronômica* **45**, 788–793 (2014).

45. Navarrete Torres, A., Velázquez Flores, J., Flores Magdaleno, H. & Pineda Pineda, J. Evaluación agronómica de cinco soluciones nutritivas sobre el desarrollo vegetativo de pitahaya (*Hylocereus undatus*) en invernadero. *Nova Scientia* **15**, 1–13 (2023).

46. De Andrés, E. F., Sánchez, F. J., Catalán, G., Tenorio, J. L. & Ayerbe, L. Vegetative propagation of Colutea istria Mill. from leafy stem cuttings. *Agroforestry Systems* **63**, 7–14 (2004).

47. Caplan, D., Stemeroff, J., Dixon, M. & Zheng, Y. Vegetative propagation of cannabis by stem cuttings: effects of leaf number, cutting position, rooting hormone, and leaf tip removal. *https://doi.org/10.1139/cjps-2018-0038* **98**, 1126–1132 (2018).

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48. Balaguera-López, H. E., Morales, E. I., Almanza-Merchán, P. J. & Balaguera L, W. A. El tamaño del cladodio y los niveles de auxina influyen en la propagación asexual de pitaya (*Selenicereus megalanthus* Haw.). *Revista Colombiana de Ciencias Hortícolas* **4**, 33–42 (2010).

49. Sarmiento-Sarmiento, G. *et al.* Auxins in the propagation of red-fleshed pitaya 'Physical Graffiti' (*Selenicereus guatemalensis* × *S. undatus*) cuttings in three substrates. *Rev. Chapingo Ser. Hortic.* **31**, 39–51 (2025).

50. Campbell, S. M., Anderson, S. L., Brym, Z. T. & Pearson, B. J. Evaluation of substrate composition and exogenous hormone application on vegetative propagule rooting success of essential oil hemp (*Cannabis sativa* L.). *PLoS One* **16**, e0249160 (2021).

51. Esteban Soto, L., Jasso Mata, J., Jesús Vargas Hernández, J., González Rosas Víctor Cetina Alcalá Ra Ximhai, H. M. & Ximhai, R. Efecto de diferentes dosis de AIB sobre el enraizamiento de *Ficus benjamina* L. en diferentes épocas del año. **2**, 795–814 (2006).

52. Cancino-García, V. J., Ramírez-Prado, J. H. & De-la-Peña, C. Auxin perception in Agave is dependent on the species' Auxin Response Factors. *Sci. Rep.* **10**, 1–11 (2020).

53. Aremu, A. O. *et al.* Physiological role of phenolic biostimulants isolated from brown seaweed *Ecklonia maxima* on plant growth and development. *Planta* **241**, 1313–1324 (2015).

54. Gaspar, T. *et al.* Plant hormones and plant growth regulators in plant tissue culture. *In Vitro Cellular and Developmental Biology - Plant* **32**, 272–289 (1996).

55. Wilson, P. J. & Staden, J. Van. Rhizocaline, Rooting Co-factors, and the Concept of Promoters and Inhibitors of Adventitious Rooting - A Review. *Ann. Bot.* **66**, 479–490 (1990).

56. Deolu-Ajayi, A. O., van der Meer, I. M., van der Werf, A. & Karlova, R. The power of seaweeds as plant biostimulants to boost crop production under abiotic stress. *Plant Cell Environ.* **45**, 2537–2553 (2022).

57. Moncada, A., Vetrano, F., Esposito, A. & Miceli, A. Effects of NAA and *Ecklonia maxima* Extracts on Lettuce and Tomato Transplant Production. *Agronomy 2022, Vol. 12, Page 329* **12**, 329 (2022).

58. Pompelli, M. F. *et al.* Effect of Indole-3-butyric acid (IBA) and 3-Indoleacetic acid (IAA) on promoting roots on micropropagated *Dyckia distachya* Hassl. *South African Journal of Botany* **184**, 387–394 (2025).

59. Ludwig-Müller, J. Ácido indol-3-butírico en el crecimiento y desarrollo de las plantas. *Plant Growth Regul.* **32**, 219–230 (2000).

60. Peña-Baracaldo, J. F., Chaparro-Zambrano, H. N., Sierra, A., Rodríguez, J. & Cabezas-Gutiérrez, M. Effect of different substrates and auxins on rooting of *Leucadendron* sp. (Proteaceae). **21**, 385–393 (2018).

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61. Cunha, C., Toyoko, S. & Souza, A. Efeito de diferentes concentrações de AIB e procedências geográficas no enraizamento de estacas de paricá. *Ciência Florestal* **28**, 1282–1292 (2018).

62. Caleño-Ruíz, B. L. & Morales-Liscano, G. Propagación asexual de especies endémicas y amenazadas del género *Passiflora* en los Andes colombianos. *Colombia Forestal* **22**, 67–82 (2019).

63. Tomaz de Oliveira, M. M., Lu, S., Zurgil, U., Raveh, E. & Tel-Zur, N. Grafting in *Hylocereus* (Cactaceae) as a tool for strengthening tolerance to high temperature stress. *Plant Physiology and Biochemistry* **160**, 94–105 (2021).

64. Nobel, P. S. & De la Barrera, E. High Temperatures and Net CO2 Uptake, Growth, and Stem Damage for the Hemiepiphytic Cactus *Hylocereus undatus*. **34**, 225–231 (2002).

65. Duval, V. S., Benedetti, G. M. & Campo, A. M. Relación clima-vegetación: adaptaciones de la comunidad del jarillal al clima semiárido, Parque Nacional Lihué Calel, provincia de La Pampa, Argentina. 33–44 (2015) doi:10.14350/rig.48033.

66. Weiss, I., Mizrahi, Y. & Raveh, E. Effect of elevated CO2 on vegetative and reproductive growth characteristics of the CAM plants *Hylocereus undatus* and *Selenicereus megalanthus*. *Sci. Hortic.* **123**, 531–536 (2010).

67. de Oliveira, M. M. T. *et al.* Shade improves growth, photosynthetic performance, production and postharvest quality in red pitahaya (*Hylocereus costaricensis*). *Sci. Hortic.* **286**, 110217 (2021).

68. Casanova-Sáez, R. & Voß, U. Auxin Metabolism Controls Developmental Decisions in Land Plants. *Trends Plant Sci.* **24**, 741–754 (2019).

69. Alcantara Cortes, J. S., Acero Godoy, J., Alcántara Cortés, J. D. & Sánchez Mora, R. M. Principales reguladores hormonales y sus interacciones en el crecimiento vegetal. *Revista Nova publicación científica en ciencias biomédicas* **17**, 109–129 (2019).

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