

The influence of hormonal balance in the multiplication of two varieties of *Hylocereus spp.*

Marcel DANCI^{1*}, Simion ALDA^{2*}, Teodor CRISTEA², Ioan DAVID³, Madalina VULPE⁴, Felicia PETCU¹, Liana ALDA³, Alexandra BECHERESCU⁵, Aneta Anca DRAGUNESCU⁵

¹University of Life Sciences “King Michael I” from Timisoara, Department of Genetic engineering, e-mail: marceldanci@usvt.ro; chilibon.felicia.fita@usvt.ro

²University of Life Sciences “King Michael I” from Timisoara, Department of Forestry, e-mail: simion_alda@usvt.ro; teodorcristea@usvt.ro

³University of Life Sciences “King Michael I” from Timisoara, Department of Food Science, e-mail: ioandavid@usvt.ro; lianaalda@usvt.ro

⁴Politehnica University of Bucharest, Faculty of Sciences, Physical Education and Computer Science, Department of Environmental Engineering and Applied Engineering Sciences, e-mail: madalina.vulpe@upb.ro

⁵University of Life Sciences “King Michael I” from Timisoara, Department of Horticulture, e-mail: alexandrabecherescu@usvt.ro; ancadragunescu@usvt.ro

* Corresponding author: marceldanci@usvt.ro; simion_alda@usvt.ro

Manuscript received: 19 November 2025; revised: 27 November 2025; accepted: 04 December 2025

Abstract

Dragon fruit (*Hylocereus undatus*) belongs to the Cactaceae family and is a species cultivated for fruit consumption due to its rich content of vitamins, minerals and an excellent source of antioxidants (betacyanin), but also vegetable albumin, vitamins and water-soluble fibres. The best method for rapid plant multiplication is cell and tissue culture, so in this study we followed the influence of different growth hormones on micropropagation and rooting, in two varieties of pitaya (Vietnamese White dragon fruit and Red Jiana Dragon Fruit). Thus, the shoots obtained from the seeds were grown on Murashige and Skoog (MS) culture medium supplemented with two types of cytokinins: 6-benzyl amino purine (BAP) – 2.5 mg/l and kinetin (KIN) – 2 mg/l, respectively naphthaleneacetic acid (NAA) – 1 mg/l, for multiplication, and for the rooting of the shoots, Murashige and Skoog (MS) medium supplemented with 1 mg/l IBA, respectively the medium without growth regulators, was used. The results obtained were different depending on the variety, but also on the type of growth regulators used.

Keywords: pitaya; micropropagation; Murashige and Skoog; growth regulators, culture media, *in vitro* culture

Introduction

Dragon fruit (*Hylocereus spp.*), commonly known as pitaya, belongs to the family *Cactaceae* and is widely cultivated in tropical and subtropical regions for its attractive and nutritious fruits. Among the cultivated species, *Hylocereus undatus* (white-fleshed pitaya) and *Hylocereus costaricensis* or *Hylocereus polyrhizus* (red-fleshed pitaya) are the most economically important. These fruits are valued for their high content of vitamins (especially vitamin C), minerals, dietary fibers, and natural antioxidants such as betacyanins and betaxanthins, which contribute to their functional and nutraceutical properties [3;12]. Due to the increasing global demand for exotic and health-promoting fruits, pitaya has gained significant attention as a high-value crop.

Conventional propagation of *Hylocereus* species is usually achieved through stem cuttings. However, this method is limited by the availability of planting material and may increase the risk of transmitting pests or diseases. In contrast, *in vitro* culture techniques offer an efficient alternative for large-scale propagation, enabling the production of genetically uniform and pathogen-free plantlets in a relatively short period of time [2;13]. Micropropagation also facilitates the conservation of germplasm and the establishment of elite clones with desirable agronomic traits.

Previous studies have demonstrated that the success of *in vitro* regeneration in *Hylocereus* depends largely on the composition of the culture medium and the type and concentration of plant growth regulators (PGRs) used [4;8]. Cytokinins such as 6-benzylaminopurine (BAP) and kinetin (KIN)

are known to stimulate axillary shoot proliferation, while auxins like indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) promote rooting and elongation. However, the optimal hormonal balance may vary depending on the genotype, explant source, and culture conditions.

Considering the economic and biological importance of pitaya, the present study aimed to evaluate the effects of different growth regulators on the *in vitro* propagation and rooting of two *Hylocereus* varieties—Vietnamese white dragon fruit (*Hylocereus undatus*) and red Jiana pitaya (*Hylocereus costaricensis*). The results obtained provide valuable information for optimizing micropropagation protocols for these species and contribute to their commercial and conservation-oriented cultivation.

Materials and Method

Plant material and seed sterilization

Seeds of two *Hylocereus* varieties — Vietnamese white dragon fruit (*Hylocereus undatus*) and red Jiana pitaya (*Hylocereus costaricensis*) — were extracted from commercially purchased ripe fruits. The seeds were washed thoroughly with distilled water to remove pulp residues and subjected to surface sterilization using 0.1% mercuric chloride (HgCl₂) for 1.5 minutes. The sterilization step was followed by three consecutive rinses with sterile distilled water to eliminate any traces of disinfectant. All aseptic manipulations were performed under a laminar airflow cabinet.

Germination and establishment of explants

Sterilized seeds were inoculated on Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) devoid of plant growth regulators, solidified with 0.8% agar and adjusted to pH 5.8 before autoclaving. Cultures were maintained in a growth chamber at 22 ± 2 °C under a 16-hour light / 8-hour dark photoperiod, using cool white fluorescent light with an intensity of approximately 2000 lux. Seed germination occurred within 10–15 days, and the obtained plantlets were used as a source of explants for subsequent multiplication experiments.

Shoot multiplication

Nodal segments and apical shoots derived from *in vitro* seedlings were cultured on MS medium supplemented with two cytokinins: 6-benzylaminopurine (BAP) at 2.5 mg/L and kinetin (KIN) at 2.0 mg/L, in combination with 1 mg/L naphthaleneacetic acid (NAA). The cultures were maintained under the same environmental conditions as described above. Subcultures were performed every four weeks to ensure continuous proliferation and to prevent nutrient depletion. Data were recorded on the number of shoots per explant, shoot length, and overall multiplication rate.

Root induction

For rooting, regenerated shoots were transferred to MS medium supplemented with 1 mg/L indole-3-butyric acid (IBA) or to hormone-free MS medium used as a control. The cultures were incubated under identical photoperiod and temperature conditions. Root initiation and development were observed and recorded after 3–4 weeks of culture.

Experimental site

All *in vitro* experiments were conducted in the Cell Culture Laboratory of the Faculty of Engineering and Applied Technologies, University of Life Sciences “King Mihai I” from Timișoara, Romania.

Results and Discussion

Seed germination and establishment of explants

Aseptic germination of *Hylocereus* seeds was successfully achieved on hormone-free Murashige and Skoog (MS) medium under controlled growth conditions. The results revealed a high germination percentage in both tested genotypes, with distinct temporal dynamics (Table 1). The Vietnamese white dragon fruit (*Hylocereus undatus*) showed a more rapid response, with 57% germination after 4 days and almost complete germination (99%) after 7 days. In comparison, the red Jiana pitaya (*Hylocereus costaricensis*) recorded 43% germination at 4 days and 92% at 7 days (Figure 1).

Table 1. Germination rate of *Hylocereus* seeds cultured aseptically on MS medium

Genotype	Germination after 4 days (%)	Germination after 7 days (%)
Vietnamese White dragon fruit (White pitaya)	57	99
Red Jiana Dragon Fruit (Red Pitaya)	43	92

The faster germination of the white pitaya seeds may be attributed to physiological or genetic differences that influence seed coat permeability or embryo vigor. Similar genotypic variability has been reported in other studies on *Hylocereus* and related *Cactaceae* species, where the rate of germination was linked to seed age, storage conditions, and internal hormonal balance [7;10]. The results also confirm that MS medium without plant growth regulators provides an adequate nutrient environment for the germination and early development of pitaya seedlings, as reported previously for *Hylocereus polyrhizus* and *Hylocereus undatus* [1;2;11].

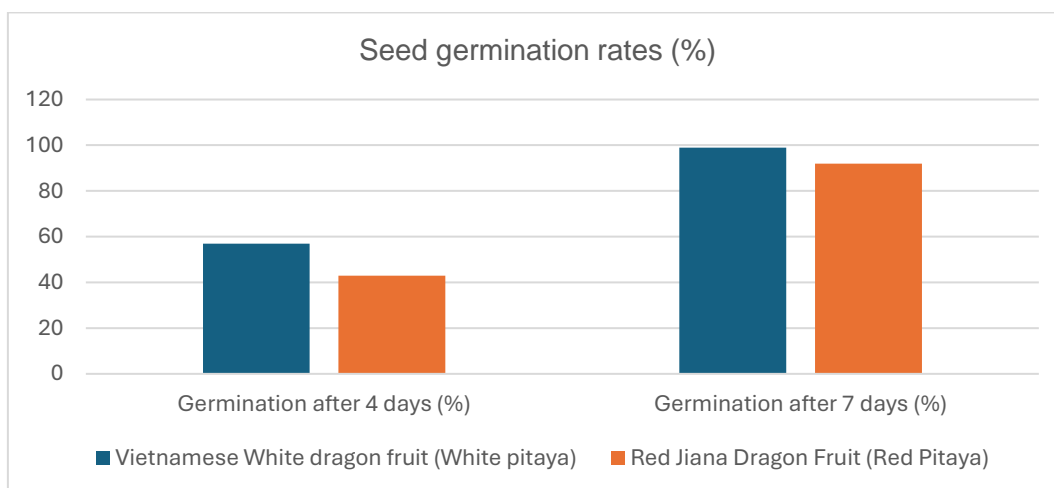


Figure 1. Comparison of aseptic seed germination rates (%) of two *Hylocereus* genotypes at 4 and 7 days of culture on MS medium without growth regulators.

These observations demonstrate that pitaya seeds maintain high viability and can be efficiently germinated *in vitro* without the need for exogenous phytohormones. The rapid and uniform germination achieved under sterile conditions ensures a reliable source of explants for subsequent micropropagation experiments.

Interpretation and literature comparison

The germination behavior observed here aligns with previous reports highlighting the suitability of hormone-free MS medium for *Hylocereus* seed germination [2;3;6]. The early emergence of radicles in the Vietnamese white variety suggests greater seed vigor, which may be correlated with differences in the composition of storage proteins or seed moisture content. According to Hernández et al. (2014), the success of *in vitro* germination in *Hylocereus* also depends on the efficiency of surface sterilization, and the present results confirm that the applied protocol (HgCl₂ 0.1% for 1.5 minutes) was effective in preventing microbial contamination.

Overall, these findings demonstrate that both genotypes are suitable for *in vitro* culture initiation, with the white pitaya showing slightly superior performance during early germination stages. This ensures the establishment of uniform plant material for subsequent stages of shoot multiplication and rooting.

Shoot proliferation

The multiplication phase of the *in vitro* experiment revealed significant differences between the two *Hylocereus* genotypes and among the two-culture media tested. The media compositions were as follows: M1: MS medium supplemented with 2.5 mg/L 6-benzylaminopurine (BAP) and 1 mg/L naphthaleneacetic acid (NAA); M2: MS medium supplemented with 2 mg/L kinetin (KIN) and 1 mg/L NAA. After 14 days of culture, the Vietnamese white pitaya (*Hylocereus undatus*) showed an average of 15.3 adventitious buds/explant on M1 and 9.7 buds/explant on M2. The red Jiana pitaya (*Hylocereus costaricensis*) produced 12.4 buds/explant on M1 and 8.2 buds/explant on M2. At 28 days, both genotypes exhibited a substantial increase in bud number, reaching 28.7 and 27.2 buds/explant on M1 for white and red pitaya, respectively, and 23.3 and 22.7 buds/explant on M2 (Table 2).

Table 2. Average number of adventitious buds obtained in the multiplication stage of two *Hylocereus* genotypes

Genotype	M1	M2	M1	M2
	(2.5 mg/L BAP + 1 mg/L NAA)	(2 mg/L KIN + 1 mg/L NAA)	(2.5 mg/L BAP + 1 mg/L NAA)	(2 mg/L KIN + 1 mg/L NAA)
	14 days	14 days	28 days	28 days
Vietnamese White dragon fruit (White pitaya)	15.3	9.7	28.7	23.3
Red Jiana Dragon Fruit (Red Pitaya)	12.4	8.2	27.2	22.7

These results indicate that the cytokinin BAP in combination with NAA (medium M1) promoted a higher rate of shoot proliferation than KIN + NAA (medium M2) in both genotypes, although the difference between genotypes became less pronounced after 28 days. The superior performance of BAP over KIN for bud induction has been reported in other *Hylocereus* species, where BAP stimulates cell division and axillary bud differentiation more effectively than kinetin [2;8].

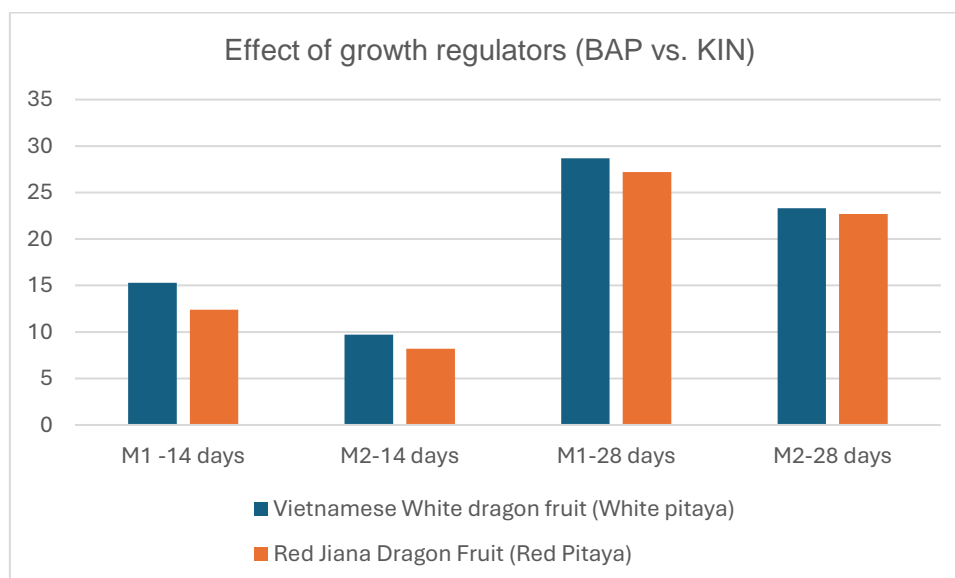


Figure 2. Effect of growth regulators (BAP vs. KIN) on the number of adventitious buds formed in two *Hylocereus* genotypes after 14 and 28 days of culture

The data presented in Figure 2 demonstrate a strong influence of both genotype and cytokinin type on the multiplication rate. Vietnamese white pitaya showed a faster initial response (at 14 days), while after 28 days, the differences between the two genotypes became minimal. The presence of BAP in the M1 medium significantly improved shoot proliferation compared to KIN, confirming that BAP acts as a more potent cytokinin for cell division and adventitious shoot formation in *Hylocereus* species. Similar findings were reported by Hernández et al. (2014) and Ramírez-Mosqueda et al. (2019), who observed that high concentrations of BAP (2-3 mg/L) in combination with low levels of NAA (0.5-1 mg/L) promoted the formation of multiple shoots with minimal callus development. Furthermore, the synergistic action between BAP and auxins such as NAA maintains apical dominance while inducing new meristematic centers, leading to efficient clonal multiplication [2;5]. Overall, these results confirm that the medium containing BAP (2.5 mg/L) + NAA (1 mg/L) is optimal for rapid *in vitro* propagation of both pitaya genotypes and provides a reliable basis for large-scale production of uniform plantlets.

Rooting

The rooting phase of *in vitro* culture was carried out on two media variants to evaluate the influence of indole-3-butyric acid (IBA) on root formation in two *Hylocereus* genotypes. The experimental variants were:

- **R1:** Murashige and Skoog (MS) basal medium without growth regulators.
- **R2:** MS medium supplemented with 1 mg/L indole-3-butyric acid (IBA).

After 14 and 28 days of culture, both genotypes exhibited efficient root induction, with minor differences between media and time intervals (Table 3). The average number of roots per plantlet increased slightly with culture duration, and the addition of IBA (R2) produced a small but consistent enhancement in root formation. The red Jiana pitaya developed a slightly higher number of roots compared with the white Vietnamese pitaya at all observation points (Figure 3).

Table 3. Mean number of roots formed in two *Hylocereus* genotypes on two media variants at 14 and 28 days

Genotype	R1 (MS without hormones)	R2 (MS + 1 mg/L IBA)	R1 (MS without hormones)	R2 (MS + 1 mg/L IBA)
	14 days	14 days	28 days	28 days
Vietnamese White dragon fruit (White pitaya)	7.3	7.9	7.5	8.1
Red Jiana Dragon Fruit (Red Pitaya)	8.5	8.7	8.6	8.9

The results indicate that both *Hylocereus* genotypes are capable of forming roots effectively under *in vitro* conditions even in the absence of exogenous auxins. However, the inclusion of IBA (1 mg/L) slightly improved root initiation and elongation in both genotypes, suggesting that the endogenous auxin levels in the explants were already sufficient to support rhizogenesis.

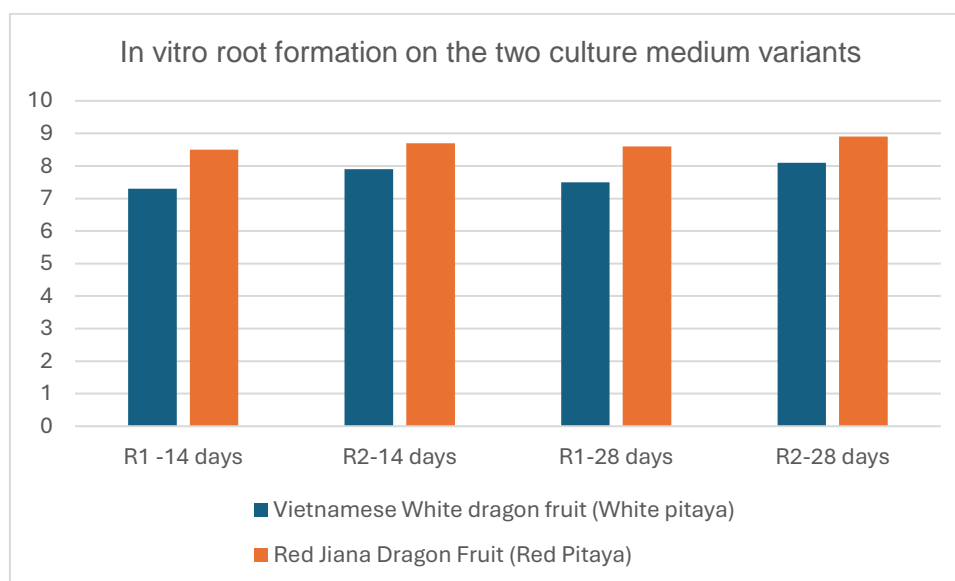


Figure 3. Effect of indole-3-butyric acid (IBA) on the mean number of roots formed in two *Hylocereus* genotypes after 14 and 28 days of culture

Comparable results were reported by Chen et al. (2016) and Ramírez-Mosqueda et al. (2019), who observed that *Hylocereus* species readily produce roots on hormone-free MS medium, yet the application of IBA enhances the number and uniformity of roots. The modest stimulatory effect of IBA in this study supports previous findings that low auxin concentrations promote root elongation without causing callus formation [4;9].

Interestingly, the red Jiana pitaya displayed slightly higher root numbers across all treatments and time intervals, which may be attributed to genotypic differences in auxin sensitivity or tissue metabolic activity. These results confirm that *Hylocereus* genotypes exhibit efficient root formation *in*

in vitro and that a low concentration of IBA can further stabilize and accelerate the process, facilitating the preparation of vigorous plantlets for acclimatization.

Conclusions

The present study demonstrated the successful *in vitro* propagation of two *Hylocereus* genotypes—Vietnamese white dragon fruit (*Hylocereus undatus*) and red Jiana pitaya (*Hylocereus costaricensis*)—through aseptic seed germination, shoot multiplication, and rooting. The results confirmed that both genotypes exhibit high viability and adaptability under controlled culture conditions.

Aseptic germination on hormone-free Murashige and Skoog (MS) medium produced rapid and uniform seedling development, providing healthy explants for further multiplication.

During the shoot proliferation stage, the MS medium supplemented with 2.5 mg/L BAP and 1 mg/L NAA (M1) induced a significantly higher number of adventitious buds compared with the kinetin-based medium (M2). The presence of BAP proved to be the most effective cytokinin for promoting axillary bud differentiation and maintaining stable growth.

In the rooting stage, both genotypes formed roots successfully on MS medium with or without the addition of IBA, indicating a high intrinsic rooting potential. However, supplementation with 1 mg/L IBA slightly improved root number and uniformity, especially in the red Jiana variety.

Overall, the established micropropagation protocol is efficient, reproducible, and suitable for large-scale production of uniform, disease-free *Hylocereus* plantlets. These results contribute valuable information for the optimization of propagation systems and genetic conservation programs in dragon fruit species, supporting their commercial expansion and sustainable cultivation.

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