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Gibberellic acid (GA_3) in the germination and in vitro development of *Hylocereus polyrhizus*

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ABSTRACT

This study aims to determine the optimal dose of gibberellic acid (GA_3) in modified MS medium for the in vitro germination and development of red-fleshed dragon fruit explants (*Hylocereus polyrhizus*). Seeds from mature, red-fleshed fruits were inoculated in modified MS medium with GA_3 doses of 0.0, 0.25, 0.50, 0.75, and 1.0 mg L^{-1}, and germination percentage was evaluated weekly. Sixty days after inoculation, explant height, longest root length, longest shoot length, and average number of roots were assessed. The 1.0 mg L^{-1} dose resulted in the highest germination percentage, greater cladode height, and longer shoot length. In contrast, root length and average number of roots were favored in the absence of GA_3. It is concluded that GA_3 application influences germination and early growth, with 1.0 mg L^{-1} being the most effective dose to accelerate germination and aerial growth. At the same time, the absence of the hormone promotes root development.

Keywords: *Cactaceae*, Gibbelellin, Micropropagation, Dragon Fruit.

Ácido giberélico (GA_3) na germinação e desenvolvimento in vitro de *Hylocereus polyrthizus*

RESUMO

Objetivou-se determinar qual a melhor dosagem de GA_3 (ácido giberélico) em meio MS modificado para germinação e o desenvolvimento in vitro dos explantes de pitaia polpa vermelha (*Hylocereus polyrhizus*). Foram utilizadas sementes de pitaia de polpa vermelha, retiradas de frutos maduros, inoculadas em meio MS modificado com as doses de 0,0 - 0,25 - 0,50 - 0,75 e 1,0 mg L^{-1} de GA_3. Avaliou-se semanalmente a porcentagem de germinação. Aos 60 dias após a inoculação, foram avaliados a altura dos explantes, o comprimento da maior raiz, o comprimento da maior brotação e número médio de raízes. A dose de 1,0 mg L^{-1} proporcionou a maior percentagem de germinação das sementes, além de promover uma maior altura do cladódio desenvolvido e comprimento da maior brotação. Em relação ao comprimento da maior raiz e número médio de raízes a não adição do GA_3 no meio de cultura propicia um melhor resultado. Conclui-se que a aplicação de GA_3 influencia a germinação e o crescimento inicial, sendo 1,0 mg L^{-1} a dose mais eficiente para acelerar a germinação e o crescimento aéreo, enquanto a ausência do hormônio favorece o desenvolvimento radicular.

Palavras-chave: *Cactaceae*, Giberelina, Micropropagação, Fruta-do-Dragão.

1. Introduction

Currently, there is a growing interest among producers and the general population in the cultivation of exotic fruits, such as dragon fruit (*Selenicereus costaricensis* (syn. *Hylocereus polyrhizus*)), also known as pitaya, due to its vivid coloration and the spherical shape of its fruits, which are composed of numerous scales known as bracts (Quiroz–González et al., 2018; Costa Júnior et al., 2023).

One of the advantages of cultivating dragon fruit orchards is related to the hardiness of the plants and their wide adaptability to diverse edaphoclimatic conditions. Additionally, their fruits have high commercial value (Trindade et al., 2023). Another benefit of dragon fruit is that its fruits have high nutritional value due to the presence of bioactive compounds (Ribeiro et al., 2021).

However, for successful dragon fruit production, attention must be given to cultivation practices to obtain quality seedlings. The commercial production of dragon fruit seedlings is typically done using stem cuttings obtained from pruning leftovers, commonly known as cladodes (Suárez et al., 2014; Neto et al., 2024). Nonetheless, a disadvantage of this propagation method is the risk of disease and pest transmission due to contamination and variability in rooting and growth capacity (Gonçalves et al., 2020).

The production of dragon fruit seedlings via *in vitro* culture presents benefits such as a high quantity of propagated material in a reduced space and high-quality seedlings. However, for the *in vitro* development of material (seeds, explants) to occur properly, it is necessary to perform appropriate procedures, including surface sterilization of explants and maintaining aseptic conditions in the laboratory, and choose the appropriate culture medium (Bozkurt et al., 2022). One medium that can be used is Murashige and Skoog (MS) (Murashige and Skoog, 1962), supplemented with plant growth regulators such as gibberellins.

In a study evaluating the *in vitro* germination of white-fleshed dragon fruit (Ferreira et al., 2007), the authors observed that gibberellins (GA_3) can enhance the germination potential and initial development of the seeds. This behavior may be justified by the role of gibberellic acid in assisting physiological responses such as cell elongation and induction of seed germination and enzyme production during germination (Arruda et al., 2019).

In another experiment assessing the effect of GA_3 doses on the germination potential in different maturation stages of white-fleshed fruits (Neta et al., 2022), the authors found that using GA_3 can increase the germination percentage of mature fruit seeds. However, few studies have determined the optimal GA_3 concentration for red-fleshed dragon fruit seeds.

Therefore, this study aimed to evaluate the effect of different concentrations (0.0, 0.25, 0.50, 0.75, and 1.0 mg L^{-3}) of gibberellic acid (GA_3) on seed germination and their *in vitro* development.

2. Material and Methods

The experiment was conducted at the micropropagation laboratory of the Federal Institute of Education, Science and Technology of Southeast Minas Gerais, Barbacena Campus (IF Sudeste MG – Campus Barbacena).

Seeds from ripe red-fleshed dragon fruit (*Selenicereus costaricensis* (syn. *Hylocereus polyrhizus*)) were used. These fruits were obtained from experimental orchards at the Federal University of Lavras (UFLA).

The fruits were cut transversely to remove the pulp along with the seeds. To extract the seeds, the pulp was washed under running water through a 2 mm nylon sieve to remove any pulp residue. The seeds were then placed in a container with 40 mL of H_2O and 40 mL of 1 mol L^{-1} HCl for 50 minutes to remove the mucilage. After this period, the seeds were rinsed under running water, dried in the shade for 48 hours, stored in Kraft paper bags, and kept in a refrigerator.

The culture medium used was a modified Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), in which ammonium nitrate (NH_4NO_3) was replaced by ammonium sulfate [$(NH_4)_2SO_4$] and calcium nitrate [$Ca(NO_3)_2H_2O$], as recommended by Ribeiro et al. (2021). The medium was supplemented with 15 g L^{-1} of sucrose and 6 g L^{-1} of bacteriological agar as the gelling agent. The treatments consisted of the following GA_3 concentrations: T1: 0.0 mg L^{-1} GA_3; T2: 0.25 mg L^{-1} GA_3; T3: 0.50 mg L^{-1} GA_3; T4: 0.75 mg L^{-1} GA_3; T5: 1.0 mg L^{-1} GA_3. The pH of the culture media was adjusted to 5.8 before adding the agar.

After preparation, 25 mL of medium was dispensed into test tubes (15 cm height × 2.0–2.5 cm diameter), which were then sealed and autoclaved at 120 °C for 20 min under 1.05 kg cm^{-2} pressure.

Under aseptic conditions in a laminar flow hood, seeds were surface-sterilized before inoculation by immersing them in 70% alcohol for 1 minute, followed by a 0.5% sodium hypochlorite (NaClO) solution with two drops of Tween 20 for 10 minutes with constant agitation. The seeds were then rinsed three times with distilled, deionized, and autoclaved water to remove excess sterilizing agents.

One dragon fruit seed was inoculated into each test tube containing the culture medium with different treatments. After inoculation, the tubes were transferred to a growth room with a 16-hour light

cycle, temperature of 25 ± 2 °C, and light intensity of 27 μmol m^{-2} s^{-1} for 60 days.

Germination assessments were conducted weekly, up to 60 days after seed inoculation, recording the number of seeds germinated in each period. Based on these data, the germination speed index (GSI) was determined according to the formula proposed by Maguire (1962) (eq 1):

$$GSI = \sum \frac{n_i}{t_i} \quad (eq\ 1)$$

where n_i is the number of seeds germinated in week i, and t_i is the time (in days) from sowing to the corresponding count.

The mean germination time (MGT) was also calculated, following Labouriau (1983), using the expression (eq 2):

$$GSI = \sum \frac{ni\,.\,ti}{ti} \quad (eq\ 2)$$

where the numerator corresponds to the sum of the product between the number of germinated seeds and the time in days of each count, and the denominator corresponds to the total number of seeds germinated during the evaluation period.

The germination percentage of the seeds was evaluated weekly until germination stabilized, using the following formula (eq 3):

$$GP = \frac{N}{A} * 100 \quad (eq\ 3)$$

GP = Germination percentage.

N = Total number of germinated seeds at the end of the experiment.

A = Total number of inoculated seeds.

At 60 days after inoculation, the seedlings were evaluated for their development in terms of seedling height, length of the longest shoot (cm), number of roots, and length of the longest root (cm).

The experimental design was a randomized block design with five GA_3 doses and five replicates, each replicate consisting of 25 seeds. Data were analyzed by regression using Sisvar software, version 5.8 (Ferreira, 2011).

3. Results and Discussion

The germination percentage curves for dragon fruit seeds as a function of GA_3 concentration (Figure 1A) indicate that at seven days after sowing, more than 50% of the seeds germinated in the MS medium supplemented with 0.25 mg L^{-1} GA_3. In contrast, no germination was observed at 0.75 mg L^{-1}, beginning only after 14 days. Germination varied over the following weeks until stabilization was reached. For 0.50 mg L^{-1} and 1.0 mg L^{-1}, stabilization occurred at 21 days; for 0.25 mg L^{-1}, at 28 days; and for 0.0 mg L^{-1} and 0.75 mg L^{-1}, at 35 days after sowing.

The germination speed index (GSI) of dragon fruit seeds (Figure 1B) was significantly influenced by GA_3, with higher values observed at intermediate concentrations. In contrast, both very low and very high concentrations reduced the GSI. These results indicate a positive effect of GA_3 on germination speed, with the most efficient response obtained at moderate concentrations.

The mean germination time (MGT) (Figure 1C) decreased with increasing GA_3 concentration, reaching the lowest value at 1.0 mg L^{-1} concentration. This finding demonstrates that the highest concentration of GA_3 promoted faster germination, while lower concentrations were less effective, resulting in longer germination times.

In a study evaluating the effects of different GA_3 concentrations (0 – 50 – 100 – 500 mg L^{-1}) based on the maturation stage of white-fleshed dragon fruit (Neta et al., 2022), the authors observed that five days after seed inoculation, germination varied from 35.5% at 0 mg L^{-1} concentration to a maximum germination rate of 37.25% at 500 mg L^{-1}. After ten days, germination increased, with the 50 mg L^{-1} concentration resulting in the highest number of germinated seeds (43.25%), whereas the control treatment (without GA_3) exhibited the lowest germination rate (41.75%). These results contradict those of the present study.

A growing number of studies have investigated the use of gibberellic acid (GA_3) in seed germination across different plant species, aiding their growth (Shah et al., 2023). This can be explained by the fact that exogenous application of GA_3 may favor or inhibit seed germination depending on the plant species, as this hormone regulates protein and RNA synthesis, thereby influencing seed reserve mobilization and embryo development (Da Silva and Leonel, 2017).

Gibberellins play a key role in seed germination by overcoming dormancy and mobilizing reserves by synthesizing α-amylase. This phytohormone influences protein metabolism by regulating reserve tissue hydrolysis in the embryo. When appropriately applied to seeds, GA_3 promotes cell elongation, enabling the primary root to break through the tissues that restrict its growth (Taiz et al., 2021). The acceleration and uniformity of germination are associated with appropriate concentrations of exogenous GA_3 applications (Ge et al., 2023).

Although final germination percentages were similar between the control and the highest GA_3 concentration, the reduced time required for germination stabilization highlights an important physiological effect of gibberellins.

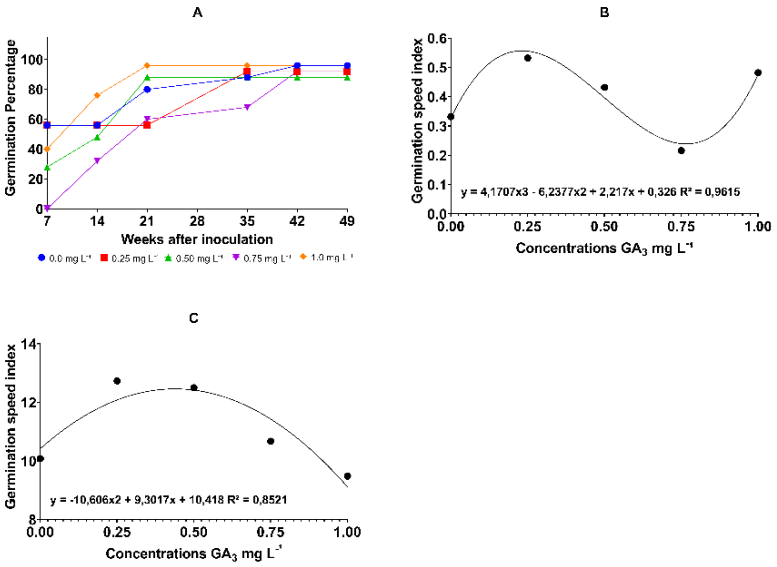

Figure 1. Seed germination (A), germination speed index (B), and mean germination time (C) of red dragon fruit over the weeks after sowing in culture medium supplemented with different GA_3 concentrations.

The acceleration of the germination process observed with 1.0 mg L^{-1} GA_3 suggests that this treatment promotes faster embryo activation and mobilization of seed reserves, processes known to be regulated by gibberellins (Taiz et al., 2021).

Analysis of variance revealed significant differences at the 1% and 5% probability levels among the tested GA_3 concentrations for the evaluated parameters. The results are presented in Table 1.

For germination percentage (Figure 2A), the regression curve showed a quadratic trend, with the highest concentration (1.0 mg L^{-1}) resulting in the greatest germination percentage, followed by 0 mg L^{-1} (92%). The 0.5 mg L^{-1} concentration produced the lowest germination (60%) compared to the others.

In Figure 2B, GA_3 concentrations influenced cladode height, with the regression curve also displaying a quadratic response. The 1.0 mg L^{-1} concentration resulted in the greatest height (4.28 cm), while the 0.5 mg L^{-1} dose showed the lowest result among the tested concentrations (1.08 cm). For shoot length (Figure 2C), GA_3 concentrations affected shoot development. The highest concentration (1.0 mg L^{-1}) resulted in the greatest shoot length (3.64 cm), whereas 0.5 mg L^{-1} produced the shortest shoots (0.84 cm).

For the average number of roots (Figures 2D and 2E), the regression model showed a quadratic response, where the absence of GA_3 (0 mg L^{-1}) resulted in the highest average number of roots (7.6). In contrast, the 0.5 mg L^{-1} produced the lowest average number of roots (3.2) among treatments. For the length of the longest root (Figure 3B), the absence of GA_3 in the culture medium resulted in the longest root (6.76 cm), whereas 0.5 mg L^{-1} produced the shortest root (3.5 cm).

In a study aimed at improving the germination of *Hylocereus undatus* seeds using GA_3 concentrations of 0, 10, 25, 50, 75, and 100 mg L^{-1} (Anagha et al., 2024), the authors observed that after 60 days, the highest GA_3 concentration resulted in the greatest seed germination percentage (87%).

In contrast, the 0 mg L^{-1} concentration exhibited the lowest germination rate (68%). In a study evaluating the effect of a 5-minute immersion of dragon fruit seeds in different GA_3 concentrations (0 - 50 - 100 - and 500 mg L^{-1}) (Neta et al., 2022), the highest germination percentage (93%) was obtained with the 100 mg L^{-1} concentration. This is consistent with the current study, where the highest GA_3 concentration led to a higher germination percentage.

Table 1. Analysis of variance for germination percentage (GSP), cladode height (CLAD HT), length of the longest root (LLR), average number of roots (ANR), and shoot length (LLS) of red dragon fruit seeds cultured in medium supplemented with different GA_3 concentrations

Source of variation	Mean square				
	GSP	CLAD HT	LLR	ANR	LLS
Concentrations	1376.000000**	9.052600*	17.818600*	26.159600*	5.746400*
Error	344.000000	1.922600	0.731600	0.379600	0.880800
CV (%)	2.95	2.28	1.45	3.31	1.28
Average	80.80	2.65	3.98	4.62	1.94

*Significant at the 5% probability level. Significant at the 1% probability level.

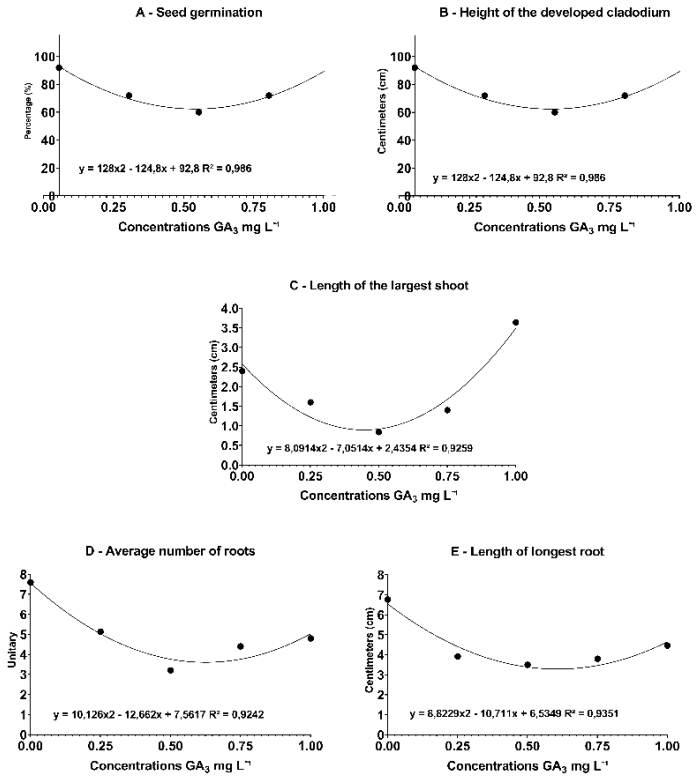

Figure 2. Seed germination percentage (A); cladode height (cm) (B), length of the longest shoot (cm) (C), average number of roots (D), and length of the longest root (cm) (E) of red dragon fruit grown in culture medium supplemented with different GA_3 concentrations.

One possible explanation for GA_3 influencing the germination potential of red dragon fruit seeds may be related to the hormone's role in modulating the germinative process (Sarwar et al., 2023). In this study, germination rates ranged from 60% to 92%. This high germination potential may be attributed to the *in vitro* culture conditions, which provide controlled temperature, light, and a nutrient-rich medium that supports optimal seed germination (Santos et al., 2022).

In a study assessing the effect of GA_3 on cladode height and shoot length of in vitro-grown seedlings after 90 days (Anagha et al., 2024), the authors concluded that GA_3 directly influences these parameters. The highest concentration used (100 mg L^{-1}) resulted in greater cladode height (18.33 cm) and shoot length (10.27 cm), which aligns with the findings of the present study.

This may be explained by the fact that GA_3 participates in the development of vegetative organs. Its mechanism of action includes increasing the number and elongation of leaf blades (Cezar et al., 2015). At an optimal concentration, it activates the synthesis of enzymes such as xyloglucan endotransglucosylase, promoting cell wall loosening and plant growth (Almeida and Rodrigues, 2016; Taiz et al., 2021).

In a study with white dragon fruit seedlings cultured with different GA_3 concentrations via micropropagation (Gonçalves and Rodrigues, 2016), the authors observed a decrease in the average number of roots as GA_3 concentration increased.

One explanation for this phenomenon is that gibberellin is a plant hormone that supports germination, cell elongation, and division but has a limited role in root growth (Taiz et al., 2021). When higher concentrations are added to the culture medium, they may cause hormonal imbalances, reducing or inhibiting root formation (Shi et al., 2024). This occurs because the signal transduction required to stimulate gibberellin-associated growth does not manifest effectively in roots, resulting in a suppressive effect of gibberellins on rooting. This inhibition may be due to the stimulation of vegetative growth, which competes with root formation (Lakehal and Bellini, 2019; Awotedu et al., 2021).

4. Conclusions

The application of gibberellic acid (GA_3) significantly affects the germination and early growth of dragon fruit seeds (*Hylocereus polyrhizus*).

The 1.0 mg L^{-1} concentration accelerates germination, shortens the time required for stabilization, and promotes greater cladode height and increased main shoot length, whereas root development is favored in the absence of GA_3.

Authors' Contribution

All authors contributed equally to this manuscript**.** Lucas Augusto Tarcísio da Silva, Carlos Henrique Milagres Ribeiro, Marília Maia de Souza, Lorena Lopes Ferreira and Leila Aparecida Salles Pio, performed data analysis, writing, and revision. Carlos Henrique Milagres Ribeiro, Lucas Augusto Tarcísio da Silva, Marília Maia de Souza and Leila Aparecida Salles Pio conducted text editing. Lucas Augusto Tarcísio da Silva, Carlos Henrique Milagres Ribeiro and Lorena Lopes Ferreira conducted the experiment and data collection.

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