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Original Article

Effects of gibberellic acid concentration and fruit maturation stage on seed germination and vigor of pitahaya seedlings

Doses de ácido giberélico e estágio de maturação de frutos na germinação de sementes e vigor de plântulas de pitaya

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Abstract

With increased interest in cultivation, the study of white-fleshed pitahaya (*Selenicereus undatus* (Haw.) D.R. Hunt, Cactaceae family) seedling production is of fundamental importance in the search for novel techniques to increase cultivation and guarantee homogeneous and productive orchards. The present study investigated the influence of various gibberellic acid (GA3) concentrations and fruit maturation stages on seed germination and vigor of white-fleshed pitahaya seedlings, considering the physiological quality of seedlings produced to support genetic breeding and conservation programs of the species. White-fleshed pitahaya seeds at two maturation stages (physiologically ripe and maintained at 10 °C in Biochemical Oxygen Demand incubators for three months) were treated with varying GA3 concentrations of 0, 50, 100, and 500 mg/L. We observed the influence of fruit storage on seedling germination, emergence, and growth as a function of GA3 concentration. According to the results, seeds extracted from ripe white-fleshed pitahaya fruits grown under the conditions tested here required GA3 application to increase seedling emergence and vigor, with optimal doses in the 150–300-mg/L range. In the case of pitahaya fruits intended for storage for future seed removal and maintained under the same sowing conditions, the application of higher doses of GA3 was necessary when compared to the previous condition, with a minimum dose of 500 mg/L GA3. The present study shows that the maturation stage of white-fleshed pitahaya fruits intended for seed removal influences the quality of seedlings; therefore, the use of seeds extracted from ripe pitahaya fruits without fermentation is more appropriate for the purpose.

Keywords: *Selenicereus undatus* (Haw.) D.R. Hunt, white-fleshed pitahaya, fruit storage, growth regulator, seedling production.

Resumo

Com o crescente interesse no seu cultivo, estudos na produção de pitaya ((*Selenicereus undatus* (Haw.) D.R. Hunt, família Cactaceae) é de fundamental importância quando se busca novas técnicas para o incremento do cultivo e garantia de um pomar homogêneo e produtivo. Objetivou-se avaliar a influência do uso de diferentes concentrações de ácido giberélico e do estágio de maturação de frutos na germinação de sementes e vigor de plântulas de pitaya vermelha da polpa branca, tendo em vista a qualidade fisiológica de mudas produzidas a fim de subsidiar trabalhos de melhoramento genético e conservação da espécie. Foram utilizadas sementes de frutos de pitaya vermelha da polpa branca em dois estágios de maturação (fisiologicamente maduros e conservados à 10°C em BOD por 3 meses), tratadas com diferentes concentrações de ácido giberélico: 0, 50, 100 e 500 mg/L. Foi possível observar que houve influência do armazenamento de frutos na germinação, emergência e crescimento das plântulas em função das concentrações de ácido giberélico. Assim, verifica-se que, no caso de sementes extraídas de frutos maduros de pitaya vermelha da polpa branca, quando semeadas nas condições do presente estudo, é necessário a utilização de GA3 para incrementar a emergência e o vigor das plântulas, com doses ótimas variando entre 150 e 300 mg/L. No caso da necessidade em armazenar os frutos de pitaya para a posterior retirada de sementes, também nas mesmas condições de semeadura do presente estudo, observa-se que, em síntese, é necessária a utilização de GA3, porém em doses maiores do que o observado no primeiro, sendo de, no mínimo, 500 mg/L. O presente estudo mostra que há influência do estágio de maturação dos frutos de pitaya vermelha de polpa branca para retirada de sementes sobre a qualidade das mudas, concluindo que o uso de sementes extraídas de frutos maduros, sem fermentação, é mais adequado para esta finalidade.

Palavras-chave: *Selenicereus undatus* (Haw.) D.R. Hunt, pitaia vermelha de polpa branca, armazenamento de frutos, regulador de crescimento, produção de mudas.

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1. Introduction

White-fleshed pitahaya (*Selenicereus undatus* (Haw.) D.R. Hunt) is a species belonging to the Cactaceae family and is native to tropical and subtropical America. The species is considered a promising fruit crop for cultivation owing to the increased consumption of exotic fruits, commercial value, rusticity, and medicinal properties (Marques et al., 2011).

The search for high-quality fruits with high market value should be encouraged, with seedling production being the first step (Fernandes and Coutinho, 2019); therefore, improving the initial stage is of fundamental importance in the search for new techniques to increase pitahaya cultivation and production.

Commercially, asexual clonal propagation using stem cuttings is generally the preferred mode of reproduction in *Hylocereus* spp. (Bellec et al., 2006) or *in vitro* cultivation (Hua et al., 2015). However, the use of seeds is important for conventional breeding and conservation of plant genetic resources (Ortiz-Hernández and Carrillo-Salazar, 2012). Little or no information is available with regard to the factors that influence the germination and storage of cactus seeds, particularly those from plants belonging to the genus *Hylocereus* (Suárez-Román et al., 2012).

The sexual reproduction method provides the necessary genetic variability for the selection of desirable traits in a breeding program, in addition to enabling scientific investigation of factors that influence germination biology, as well as the preservation of the diversity of phytogenetic resources and species conservation through germplasm banks (Ruths et al., 2019).

Pitahaya seeds have been investigated for the propagative characterization of the species and such studies should be initiated at the fruit maturation stage, which can influence the physiological quality of seeds and, consequently, seedling production (Santos et al., 2018). Despite being considered non-climacteric (Ortiz and Takahashi, 2015), pitahaya fruits deteriorate relatively easily under ambient conditions, with the storage period and temperature influencing their physiological processes, in turn, increasing their shelf life (Magaña et al., 2006), which can also influence the physiological quality of seeds.

In addition, knowledge of the germination process and substances capable of improving the process are of great relevance in botanical studies, especially in the field of seedling production (Nobrega et al., 2018). In such a context, owing to its great potential in commercial and agronomic areas, studies regarding the influence of gibberellic acid (GA3) have increased significantly over the last few decades, as it promotes seed germination in several plant species, in turn, stimulating their growth (Lopes et al., 2009). Such information is beneficial for breeding programs, which select the best phenotypic and genotypic characteristics of the species to increase productivity (Andrade et al., 2008; Lone et al., 2014).

Therefore, the present study aimed to evaluate the influence of various GA3 concentrations and fruit maturation stages on the seed germination, emergence, and vigor of white-fleshed pitahaya seedlings, considering their physiological quality, to support genetic breeding and conservation programs of the species.

2. Material and Methods

Experiments were conducted at the Laboratório de Sementes do Departamento de Fitotecnia, Tecnologia de Alimentos e Sócio-Economia da Faculdade de Engenharia de Ilha Solteira-FEIS/UNESP and were divided into two stages based on the maturation stage of fruits from which seeds were extracted for the performance of tests. Ripe pitahaya fruits were purchased from a local market, standardized visually according to their color and consistency, and senescent fruits maintained at 10 °C in Biochemical Oxygen Demand incubators for three months after the previous stage, when they exhibited visible signs of deterioration (Marques et al., 2011).

In both stages, seeds were extracted through openings on the fruits. Seeds and pulp were macerated and the mixture passed through a 2-mm mesh sieve. The seeds were washed in running water and subsequently immersed in water with 2.5% sodium hypochlorite to remove the remaining mucilage. Subsequently, the seeds were packed in paper towels and placed in the shade for 48 h to dry.

The dried seeds were immersed in GA3 solutions at concentrations of 0, 50, 100, and 500 mg/L for 5 min, and the control was immersed in distilled water for the same period. After removing the excess solution, tests were performed.

2.1. Germination tests

Sowing was carried out on sheets of filter papers moistened with deionized water (control) and for the other treatments, the filter papers were moistened with the treatment solution, which was equivalent to 2.5-fold the weight of the filter paper (Brasil, 2009). The treated filter papers were packed in transparent plastic germination boxes (gerbox) with lids, with each box containing 50 seeds. The boxes were then placed in a germination chamber at a temperature of 25 °C, with subsequent rewetting as required.

The experimental design adopted was completely randomized, with four treatments (three GA3 concentrations + control) containing four replicates, considering each gerbox as one replicate and 50 seeds per replicate.

The following variables were evaluated:

First Count (FC): The percentage of normal seedlings germinated five days after sowing (DAS) was computed, and seedlings that exhibited the potential to continue their development and give rise to normal plants were considered normal based on shoot emission and root system development as the criterion (Dresch et al., 2013). FC was expressed as a percentage.

Germination (G): After seed germination stabilization (on the 15th DAS for seeds from ripe fruits and 35th DAS for seeds from senescent fruits), the number of germinated seedlings was evaluated by computing the percentage of normal seedlings using the following formula: $G (\%) = (N/50) \times 100$, where N is the number of seeds germinated at the end of the test (Brasil, 2009). Germination was expressed as a percentage

Germination Speed Index (GSI), which was calculated using the formula GSI = ∑(ni/ti), where ni is the number of seeds that germinated in time 'i'; ti = time after test performance (Maguire, 1962).

Seedling length: The entire seedling length (root system and shoot) of 10 seedlings that were randomly selected from the germination test was measured using a graduated rule. Measurements were expressed in centimeters (cm).

Total fresh weight: Ten whole seedlings, as described previously, were weighed on a precision scale of 0.001 g (1 mg) to obtain the total fresh weight of seedlings in each treatment, which was expressed in grams (g).

2.2. Emergence tests

Emergence tests were performed after the germination tests. The second batch of dry seeds extracted from ripe and senescent fruits was immersed in GA3 solutions at concentrations of 0, 50, 100, and 500 mg/L for 5 min, with the control seeds being immersed in distilled water for the same period. After removing the excess solution, emergence tests were performed.

The tests were conducted at the Laboratório de Sementes do Departamento de Fitotecnia, Tecnologia de Alimentos e Sócio-Economia da Faculdade de Engenharia de Ilha Solteira-FEIS/UNESP, where 60 seeds from each treatment were sown in plastic trays containing organic compost with the soil type for vegetables. Manual sprinkler irrigation was applied whenever necessary to maintain the substrate at its field capacity.

At 15 DAS until seedling emergence was stable, which occurred 35 DAS, the following evaluations were carried out:

Emergence (E): The percentage of normal seedlings was computed every seven days using the following formula: E (%) = N/60 × 100, where N is the number of seeds that emerged at the end of the test. Seedling emergence was expressed as a percentage.

Emergence Speed Index (ESI) was calculated using the following formula: ESI = ∑(ni/ti), where ni is the number of seeds that emerged in time 'i'; ti is time after the implementation of the test; i = 1 $\rightarrow$ 35 days (Labouriau, 1983).

Seedling length: The entire seedling length (root system and shoot) of five seedlings that were randomly selected from the germination test was measured using a graduated rule. Measurements were expressed in cm.

Fresh seedling mass: Five previously described whole seedlings were weighed on a precision scale of 0.001 g (1 mg) to obtain the fresh seedling mass in each treatment, which was expressed as g seedling^{-1}.

Dry seedling mass: Dry seedling mass was obtained from five dry seedlings from each treatment. The seedlings were dried in an oven maintained at 60 °C for 48 h until a constant dry mass was obtained. Afterward, the dry mass of each seedling was measured on a precision analytical scale (0.0001 g) and the results were expressed as g seedling^{-1}.

The average measurements obtained after performing Analysis of Variance (ANOVA) were compared using the Tukey's test at 5% probability. Data were analyzed using SISVAR 5.6 (Ferreira, 2014) and were adjusted to a second-order polynomial regression when a significant difference was observed.

3. Results

3.1. Germination tests

The ANOVA results obtained based on the evaluated variables of seeds extracted from ripe white-fleshed pitahaya fruits are shown in Table 1. According to the results, no statistically significant difference was observed among treatments.

Table 1. Analysis of variance for the first count of seedlings germinated at 5 days after sowing, number of normal seedlings at 10 days after sowing, seedling length, total fresh seedling mass, and germination speed index (GSI) of white-fleshed pitahaya seeds (*Selenicereus undatus* (Haw.) D.R. Hunt) submitted to different gibberellic acid (GA_3) doses extracted from ripe fruits. Ilha Solteira-SP, 2020.

Source of Variation	First Count (5 days)	Number of Normal Plants (10 days)	Seedling Length (cm)	Total Fresh Mass (g)	GSI
Treatment	Mean Square				
	$8.416667^{NS, 1}$	$1.729167^{NS, 1}$	0.515722^{NS}	0.080245^{NS}	0.268958^{NS}
Doses (mg/L)	Averages				
0	35.50	41.75	2.234	0.788	11.275
50	33.75	43.25	2.965	1.075	11.075
100	35.00	42.00	2.949	1.086	11.200
500	37.25	42.25	2.956	1.048	11.675
CV%	5.56[1]	7.96[1]	26.56	18.78	4.87
Average	35.38	42.31	2.78	1.00	11.31

^{NS}Not significant by the Tukey test at 5% probability. [1]Values transformed by the equation $\sqrt{x+0.5}$.

With regard to the initial count at 5 DAS, we observed that the average germination percentage was 71.6% (corresponding to approximately 35 seedlings) and the final germination percentage at 10 DAS for the entire experiment was approximately 85% (corresponding to approximately 42 seedlings), whereas that for the control treatment was 83.5%, which was the lowest value observed for the final count and did not differ significantly from those of the other treatments.

However, contrasting results were observed for seeds extracted from senescent fruits, and their ANOVA results and average values of variables evaluated are shown in Table 2.

Based on the fruit maturation stages, we observed that germination at 10 DAS, which is the ideal time to evaluate the final germination percentage of white-fleshed pitahaya seeds obtained from ripe fruits, was superior to that of stored senescent fruits, with general averages of 85% and 60.9%, respectively. Considering that germination occurred more gradually, there was a need to extend the germination test period, which took place at 35 DAS when seed germination had ceased and the germination percentage reached 85.8%, which was close to the value observed in the first stage of the experiment.

Regarding the final germination percentage (at 35 DAS), the regression curve could be adjusted with a significant quadratic effect and coefficient of determination (R^2) value of 99.89%, indicating that the optimal dose for the variable could be approximately 245 mg/L GA3, although the highest values were observed at a dose of 100 mg/L GA3, as illustrated in Figure 1A.

We observed a significant influence of GA3 doses applied on the total fresh weight of seedlings grown from senescent fruit seeds, with weights ranging between 1.96 g and 2.28 g for treatments with 500 mg/L and 100 mg/L GA3, respectively, which demonstrated the effect of a dose of 100 mg/L GA3.

The regression curve of fresh seedling mass could be adjusted with a significant quadratic effect and R^2 value of 94.31%, indicating that the optimal GA3 dose for the variable could be approximately 257 mg/L, although the highest values were observed at a dose of 100 mg/L GA3, as illustrated in Figure 1B.

3.2. Emergence tests

Emergence tests were performed after germination tests and the ANOVA results for variables of seeds extracted from ripe white-fleshed pitahaya fruits are shown in Table 3. According to the results, statistically significant differences were observed among the treatments for all variables analyzed, except for ESI, suggesting that, unlike the germination test, the use of GA3 was beneficial to the seedlings.

Overall, the seedling emergence percentage (39.37%, corresponding to 23.63 seedlings) of white-fleshed pitahaya seedlings was lower than the germination percentage under the same treatment conditions at the end of the evaluation period (35 DAS).

Statistically significant differences were observed in GA3 concentrations between the first count (at 15 DAS) and the last count (at 35 DAS), which allowed for the adjustment of the regression curve with significant quadratic effect and R^2 values of 89.67% and 92.33%, respectively, suggesting that the optimal dose for the variables could be approximately 158 mg/L and 179 mg/L GA3, although the highest values were observed at doses of 50 mg/L and 100 mg/L GA3, as illustrated in Figure 2A.

At 35 DAS, 50.42% emergence was observed in seedlings grown from seeds treated with 100 mg/L GA3 and the counting of emerged seedlings continued for 70 days. However, no significant difference was observed in the emergence percentage when compared to the count at

Table 2. Analysis of variance and averages for the first count of seedlings germinated at 5 and 10 days after sowing, number of normal seedlings at 35 days after sowing, seedling length, total fresh seedling mass, and emergence speed index (ESI) of white-fleshed pitahaya seeds (*Selenicereus undatus* (Haw.) D.R. Hunt) submitted to different gibberellic acid (GA_3) doses extracted from senescent fruits. Ilha Solteira-SP, 2020.

Source of Variation	**First Count (5 days)**	**Count at 10 days**	**Number of Normal Plants (35 days)**	**Seedling Length (cm)**	**Total Fresh Mass (g)**	**ESI**
Treatment	Mean Square					
	$0.496637^{NS, 1}$	$0.660024^{NS, 1}$	$0.14768^{*, 1}$	0.911283^{NS}	0.082789^{*}	5.844651^{NS}
Doses (mg/L)	Averages					
0	23.70	29.10	39.50 b	3.25	1.99 c	8.778
50	22.80	28.80	43.25 ab	3.21	2.08 b	8.676
100	29.70	37.20	45.50 a	3.09	2.28 a	10.960
500	21.90	26.70	43.25 ab	3.34	1.96 d	8.286
CV%	11.27^{1}	10.37^{1}	3.23	9.91	0.01	18.55
Average	24.53	30.45	42.88	3.22	2.074	9.175

^{NS}Not significant by the Tukey test at 5% probability. ^{1}Values transformed by the equation $\sqrt{x+0.5}$. *Significant by the Tukey test at 5% probability. Different letters in columns differ statistically from each other.

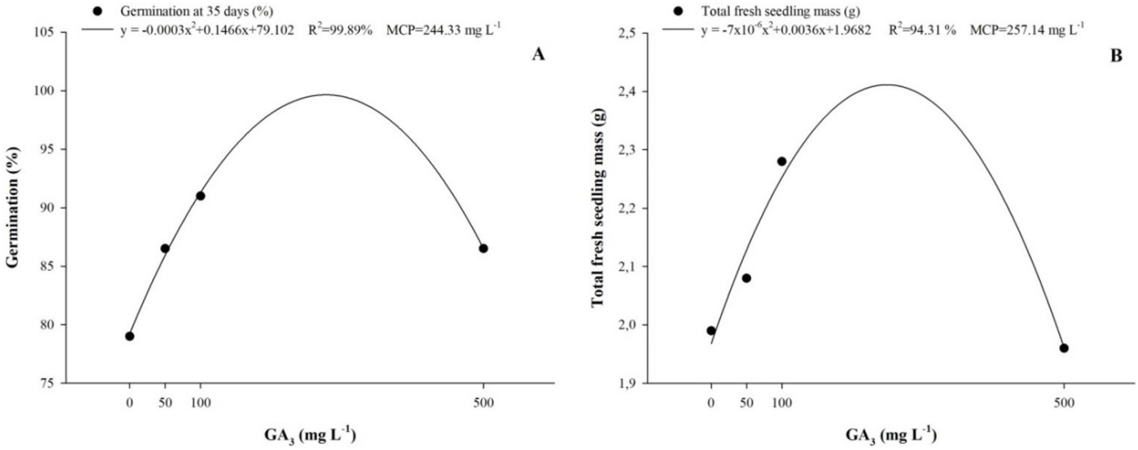

Figure 1. Germination percentage at 35 days after sowing (A) and total fresh seedling mass (B) of white-fleshed red pitahaya (*Selenicereus undatus* (Haw.) D.R. Hunt) seeds extracted from senescent fruits as a function of the gibberellic acid dose used. Ilha Solteira, 2020.

Table 3. Analysis of variance and average values for the first count of seedlings emerged at 15 days after sowing, number of normal seedlings at 35 days after sowing, seedling length, fresh seedling mass, dry seedling mass, and emergence speed index (ESI) of white-fleshed pitahaya seeds (*Selenicereus undatus* (Haw.) D.R. Hunt) submitted to different gibberellic acid (GA_3) doses extracted from ripe fruits. Ilha Solteira-SP, 2020.

Source of Variation	First Count at 15 days	Number of Normal Plants at 35 days	Seedling Length (cm)	Fresh Seedling Mass (g)	Dry Seedling Mass (g)	ESI
Treatment	Mean Square					
	2.423917*, 1	2.797057*, 1	0.911283*	0.003427*	0.000003*	1.336649NS
Doses (mg/L)	Averages					
0	11.50 ab	27.00 ab	2.103 b	0.178 b	0.005050 b	2,11
50	10.25 ab	24.50 a	2.965 a	0.156 c	0.005150 b	1.91
100	14.50 a	30.25 ab	3.110 a	0.209 a	0.006125 a	2.47
500	4.00 b	12.75 b	3.073 a	0.143 d	0.003975 c	0.91
CV%	26.36[1]	18.21[1]	14.58	0.18	3.29	37.33
Average	10.0625	23.625	2.81	0.172	0.0051	1.851

*Significant by the Tukey test at 5% probability. [1]Values transformed by the equation $\sqrt{x+0.5}$. [NS]Not significant by the Tukey test at 5% probability. Different letters in columns differ statistically from each other.

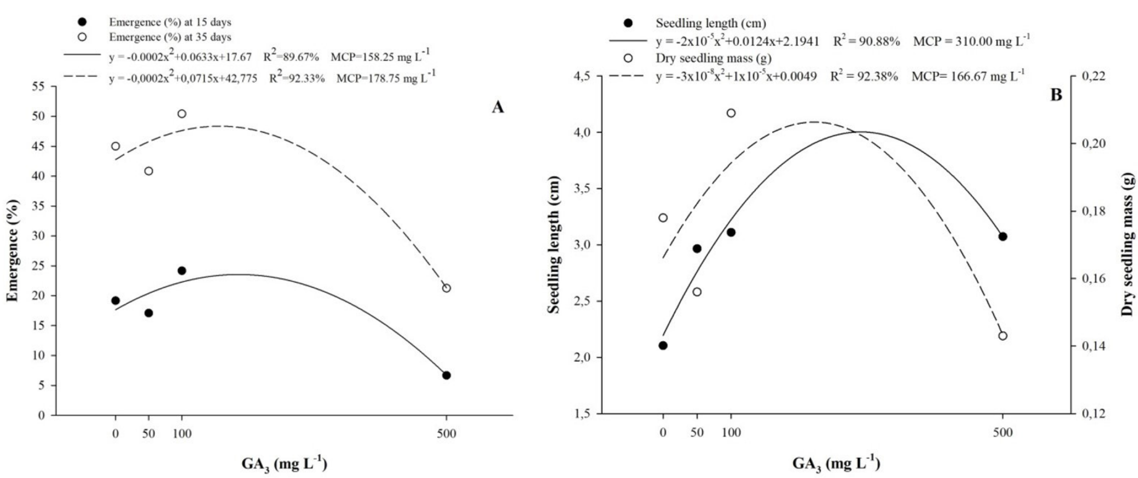

Figure 2. Emergence percentage at 15 and 35 days after sowing (A), seedling length, and dry seedling mass (B) of white-fleshed red pitahaya seeds extracted from ripe fruits as a function of GA_3 dose used. Ilha Solteira, 2020.

35 DAS, which had 50% emergence, making the additional experimental period unfeasible.

Although no statistically significant difference was observed in ESI between treatments, GA3 application influenced ESI, with the highest (2.47) and lowest (0.91) values being obtained at doses of 100 mg/L and 500 mg/L GA3, respectively. In addition, we observed that a relatively high ESI indicated rapid germination, which is beneficial for seedling production.

Regarding the physiological quality of seedlings, all variables analyzed (seedling length, total fresh weight, and dry mass) were influenced by the GA3 doses applied during seed treatment.

The average seedling length observed in the control treatment was 2.1 cm, which differed significantly from those of the other treatments, with the highest value of 3.1 cm being observed in the treatment with GA3 concentration of 100 mg/L. The results indicated a positive correlation between the use of plant regulators and seedling vigor, which allowed for the adjustment of the regression curve with a significant quadratic effect and R^2 value of 90.88%, suggesting that the optimal dose for the variables could be 310 mg/L GA3, as illustrated in Figure 2B.

With regard to the fresh and dry masses of seedlings, relatively low values (0.143 g seedling^{-1} and 0.003975 g seedling^{-1}, respectively) were observed for treatments with a dose of 500 mg/L despite the positive effect of GA3 application on seeds, and the highest values (0.209 g seedling^{-1} [fresh mass] and 0.006125 g seedling^{-1} [dry mass]) were observed at a concentration of 100 mg/L GA3. Dry mass of the highest pitahaya seedling length under the same conditions (temperature of 25 ºC) was the highest at 0.0062 g seedling^{-1}; however, a similar value was observed in seedlings grown from seeds treated with 100 mg/L GA3, indicating the influence of the plant regulator on the variables, which allowed for the adjustment of regression curves with significant quadratic effects. However, only the regression curve for the dry seedling mass had a satisfactory R^2 value of 92.38%, suggesting that the optimal dose for the variables could be approximately 167 mg/L GA3, as illustrated in Figure 2B.

The ANOVA results for variables evaluated under the emergence tests of seeds extracted from senescent white-fleshed pitahaya fruits are shown in Table 4. According to the results, statistically significant differences were observed in all variables analyzed, except for seedling length, among the various treatments with GA3.

Based on the data for the first count of emerged seedlings at 15 DAS, we observed that treatment with GA3 influenced seedling emergence, with the highest emergence value being observed in the treatment with 500 mg/L GA3, while the lowest value recorded in the control treatment, which contrasted the results of the previous test. The highest initial emergence value for seeds extracted from ripe white-fleshed pitahaya fruits was observed in the 100 mg/L GA3 treatment. However, overall, the initial averages of both experiments were similar and varied between 16% and 17%.

With regard to the final emergence percentage at 35 DAS, although the general averages between experiments (ripe fruit × senescent fruit) were almost similar (38.75% for the first emergence test and 39.63% for the second emergence test), the highest value (50%) was observed in the treatment with GA3 dose of 500 mg/L, while the same percentage was observed in the treatment with GA3 dose of 100 mg/L in the first emergence experiment, suggesting that fruit storage influences seed vigor. Therefore, a relatively high dose of the plant regulator was required to achieve the same seedling emergence percentage.

For these variables, in the case of seeds extracted from senescent fruits, the data could be adjusted to the regression curve with a significant quadratic effect, suggesting that the minimum dose required to increase emergence was

Table 4. Analysis of variance and average values for the first count of seedlings emerged at 15 days after sowing, number of normal seedlings at 35 days after sowing, seedling length, fresh seedling mass, dry seedling mass, and emergence speed index (ESI) of white-fleshed pitahaya seeds (*Selenicereus undatus* (Haw.) D.R. Hunt) submitted to different gibberellic acid (GA_3) doses extracted from senescent fruits. Ilha Solteira-SP, 2020.

Source of Variation	**First Count at 15 days**	**Number of Normal Plants at 35 days**	**Seedling Length (cm)**	**Fresh Seedling Mass (g)**	**Dry Seedling Mass (g)**	**ESI**
Treatments	Mean Square					
	0.484861^{*,1}	1.179702^{*,1}	0.852292^{NS}	0.0073*	0.000013*	0.054244^{NS}
Doses (mg/L)	Averages					
0	8.01 d	18.08 d	2.825	0.1529 d	0.0041 c	1,43 c
50	11.01 b	26.01 b	3.475	0.1975 c	0.0067 b	2,03 b
100	9.013 c	21.013 c	2.675	0.2037 b	0.0080 a	1,65 c
500	13.08 a	30.01 a	3.600	0.2572 a	0.0080 a	2,37 a
CV%	0.15^{1}	0.08^{1}	16.53	0.41	2.52	0.23
Average	10.28	23.78	3.14	0.203	0.00668	1.871

*Significant by the Tukey test at 5% probability. [1]Values transformed by the equation $\sqrt{x+0.5}$. [NS]Not significant by the Tukey test at 5% probability. Different letters in columns differ statistically from each other.

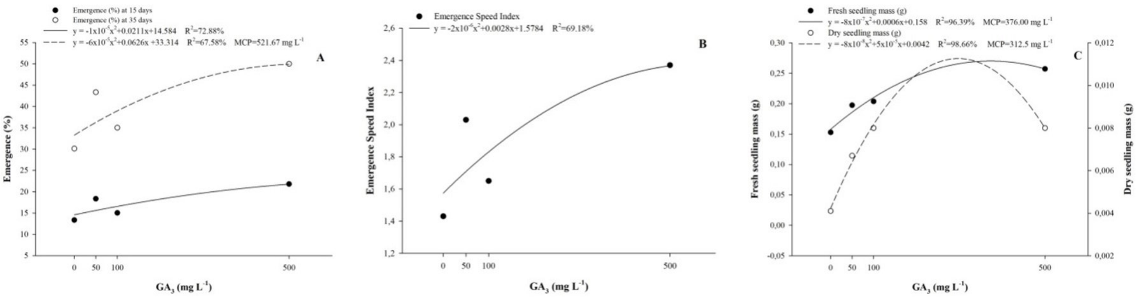

Figure 3. Emergence percentage at 15 and 35 days after sowing (A), emergence speed index (B), fresh seedling mass, and dry seedling mass (C) of white-fleshed red pitahaya seeds extracted from senescent fruits. Ilha Solteira, 2020.

higher than the maximum dose used in the present study (500 mg/L GA3), as illustrated in Figure 3A.

The ESI value of seeds extracted from ripe white-fleshed pitahaya fruits differed from that of seeds in the first emergence test and we observed that the GA3 doses applied had an effect on ESI. Statistically significant differences were observed between treatments, with the lowest ESI value (1.43) being observed in the control treatment and the highest value (2.37) in 500 mg/L GA3 treatment. The average ESI value in the second emergence test was 1.87, which was the same as that obtained in the first emergence test (1.85), demonstrating that the application of GA3 at high doses was appropriate for seeds extracted from senescent pitahaya fruits. ESI values could be adjusted to a second-order polynomial regression with R^2 value of 69.18%, indicating that the minimum dose required to increase emergence could be higher than the maximum dose applied in the present study (500 mg/L GA3), as illustrated in Figure 3B.

Among the variables evaluated, GA3 doses applied had a significant influence on fresh and dry seedling masses in the second emergence test, suggesting that an increase in GA3 concentration in the treatment of seeds was directly proportional to the highest values observed (0.2572 and 0.0080 g, respectively). The lowest values of fresh and dry seedling masses were observed in the control treatment (0.1529 g and 0.0041 g, respectively). Therefore, the data obtained could be adjusted to the regression curve with a significant quadratic effect, indicating that the minimum dose required to increase emergence could be at least 375 mg/L GA3, as illustrated in Figure 3C.

We observed that both the overall average fresh and dry seedling masses in the first emergence test were higher (0.172 and 0.0051 g, respectively, for the first emergence test; 0.203 and 0.00668 g, respectively, for the second emergence test), which confirmed the influence of GA3 on seedling emergence and vigor.

4. Discussion

4.1. Germination tests

Alves et al. (2011) evaluated the effect of different substrates on germination of white-fleshed pitahaya seeds under different temperatures and concluded that seed germination tests for the species should be performed at a constant temperature of 25 ºC on paper roll, with initial and final counts at 5 and 10 DAS, respectively. The study obtained initial and final count values of 60% and 85%, respectively, which is consistent with the values obtained in the present study.

According to Carvalho and Nakagawa (2000), the phase of maximum seed quality coincides with physiological maturation, when germination and vigor are at their maximum. Therefore, since no statistically significant difference was observed in the effect of the various GA3 concentrations on the germination of white-fleshed pitahaya seeds extracted from ripe fruits and the germination percentage was consistent with that reported in other studies, we concluded that seed treatment was not necessary for such seeds.

Because of the high sugar content in the pulp, senescent fruits tend to undergo the natural fermentation process, which is a method most commonly used to remove mucilage during the seed extraction process (Bezerra et al., 2015). However, several studies have shown that if the natural fermentation time is prolonged, biochemical transformations that alter the chemical composition of seeds can occur, in turn, reducing their quality and affecting the germination percentage, in addition to increasing seedling abnormalities (Pereira and Dias, 2000; Lima et al., 2009).

Considering the longer time required for the analysis of germination percentages, we observed that the use of GA3 to treat seeds obtained from senescent fruits exhibited statistically significant differences among the concentrations evaluated with regard to the number of normal seedlings, with averages of 39.5 (control) and 45.5 (treatment with 100 mg/L GA3), which corresponded to germination percentages of 79% and 91%, respectively. The results are consistent with the findings of Lopes et al. (2009) who observed an increase in the germination of papaya seeds following the application of GA3, with the highest values being observed at GA3 concentrations between 250 mg/L and 500 mg/L.

Similar results were obtained by Santos et al. (2016) who observed the highest initial growth in *Passiflora* spp. (Passifloraceae family) after immersion of seeds in GA3 solution. Paixão et al. (2019) investigated germination of yellow acacia seeds and observed 100% germination when seeds were treated with 3000 mg/L GA3 for 30 min. In addition, the GA3 doses applied and imbibition time were significantly higher than those used in the present study.

GA3 promotes seed germination and influences protein metabolism by controlling the hydrolysis of embryo reserve tissues. At adequate concentrations in seeds, GA3 promotes cell elongation by inducing the primary roots to break tissues that impair their growth (Taiz and Zeiger, 2013). Similarly, gibberellins influence protein metabolism by acting on the synthesis of enzymes that weaken the seed coat, which is involved in cell elongation and protrusion of the primary root.

Oliveira et al. (2010) studied the effects of GA3 on *Annona cherimola* Mill. × *Annona squamosa* L. (Annonaceae family), and Lopes et al. (2009) analyzed the effect of increasing GA3 concentrations on papaya (*Carica papaya* L.), and both researchers obtained satisfactory seedling vigor results. Santos et al. (2016) observed that seeds of *Passiflora alata* Curtis exhibited greater seedling vigor when immersed in GA3 solution at concentrations of 500 mg/L and 1000 mg/L, which is consistent with the results obtained in the present study. Furthermore, gibberellins counterbalance inhibition induced by abscisic acid, in turn, cause an endogenous increase in GA3, which demonstrates their role in overcoming seed dormancy, in addition to enhancing the physiological quality of seedlings (Carvalho et al., 2012).

Santos et al. (2013) evaluated seed germination and vigor of yellow passion fruit seedlings subjected to the action of GA and concluded that the application of GA3 at concentrations between 128 mg/L and 160 mg/L has a beneficial effect on seedling vigor.

4.2. Emergence tests

Santana et al. (2010) observed higher values in the seed germination test than in the seedling emergence test for 'pau-santo' (*Kielmeyera coriacea* Mart. & Zucc., Calophyllaceae family). Based on the results, the germination test was found to be the most appropriate to determine the physiological quality of seeds because it can be carried out in the laboratory under controlled temperature, substrate, and light conditions, allowing seeds to express maximum germination potential without undesirable external interference.

Conversely, seedling emergence testing not only depends solely on the energy contained in the endosperm or cotyledons (Hackbart and Cordazzo, 2003) but also the physical characteristics of the substrate, such as structure, aeration, water retention capacity, and degree of pathogen infestation (Albuquerque et al., 1998), in addition to temperature, humidity, seeding depth, and oxygen availability (Severino et al., 2005).

The overall seedling emergence percentage obtained in the present study was not satisfactory when compared to the findings of Lone et al. (2014) who observed that white-fleshed pitahaya seeds achieved 100% emergence in vermiculite substrate at 30 DAS and pine bark substrate had the lowest value (92%). However, Santos et al. (2018) evaluated seedling emergence at different maturation stages of white-fleshed pitahaya seeds and obtained emergence percentage values ranging between 34% and 96%.

The use of substrates that provide adequate porosity; that is, substrates that facilitate oxygen supply and water retention is crucial for seedling germination and emergence because such substrates promote vigorous seedling emergence. The soil type used in the present study may not have favored seed germination and seedling emergence when compared with other substrates used in previous studies as it was derived from household compost.

Santos et al. (2016) evaluated the influence of various GA3 doses on seedling emergence among different passion fruit species and observed that *P. alata* seedlings exhibited a relatively high seedling emergence (70.26%) at a concentration of 500 mg/L GA3, whereas *Passiflora setacea* DC., exhibited optimal seedling emergence (60.6%) when 1000 mg/L GA3 was applied. In addition, in *Passiflora gibertii* N.E. Br., a concentration of 250 mg/L GA3 achieved the best-estimated seedling emergence (81.7%). The results suggest that initial growth was directly related to the concentration of GA3 applied despite the significant variations observed in the effects of GA3 concentrations on the biometric variables in each species evaluated.

Santos et al. (2018) performed germination and emergence tests using pitahaya seeds and obtained ESI values ranging between 2.73 and 12.39, which are similar to those obtained in the present study at the lowest GA3 dose. Lone et al. (2014) evaluated ESI values of pitahaya seeds sown in different substrates and obtained values ranging between 6.44 and 17.85.

Natural fermentation of fruits during senescence can be harmful to seed germination, with an increase in the percentage of abnormal seedlings (Osipi et al., 2011). However, in laboratory tests, natural fermentation could be beneficial for seedling emergence due to aril and seed coat degradation, which facilitates water imbibition (Baskin and Baskin, 2005).

Imbibition is fundamental for germination because it allows the resumption of metabolic activities in the seed embryo, which facilitates the mobilization and assimilation of tissue reserves and subsequent embryo growth (Marcos-Filho, 2005). Furthermore, imbibition influences seedling length, fresh mass, and dry mass, which are variables that exhibited the highest values at the highest GA3 concentration (500 mg/L).

5. Conclusion

The present study has revealed that the maturation stage of white-fleshed pitahaya fruits intended for seed removal influences the quality of seedlings. Therefore, the use of seeds extracted from ripe fruits treated with optimal GA3 doses ranging from 150 to 300 mg/L of GA3 to increase seedling emergence and vigor, without fermentation, is more appropriate for the purpose. However, in the case of white-fleshed pitahaya fruits intended for storage for future seed removal, the use of GA3 at a dose of at least 500 mg/L is required.

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