Folia Hort. 35(2) (2023): 403–418

DOI: 10.2478/fhort-2023-0029

FOLIA HORTICULTURAE

Published by the Polish Society for Horticultural Science since 1989

ORIGINAL ARTICLE | Open access | https://sciendo.com/journal/FHORT

Enhanced germination of seeds native to Brazil: A comparative analysis between free and nanoencapsulated gibberellic acid in *Dyckia* sp. (Bromeliaceae)

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ABSTRACT

Brazil is home to a great diversity of species of the genus *Dyckia*. However, many of these species are threatened due to habitat destruction and predatory exploitation. An alternative to conserving these plants is sexual propagation using plant regulators to stimulate germination. Gibberellic acid (GA_3) is an effective regulator in this process, but its instability and ease of degradation pose challenges. Therefore, nanoencapsulation of GA_3 could be used to protect the molecule and allow controlled release. In this study, the effects of different doses of GA_3 were evaluated on the germination of four species: *D. cabrerae*, *D. dusenii*, *D. pottiorum* and *D. walteriana*. The first stage consisted of soaking the seeds in different concentrations of GA_3, in which the species *D. dusenii* and *D. walteriana* showed significant responses to GA_3, with an increase from 35% to more than 60% germination. However, the species *D. cabrerae* and *D. pottiorum* responded positively to GA_3 only in vegetative growth parameters. In the second stage, the use of nanoparticles of alginate/chitosan (NP ALG/CS) and chitosan/tripolyphosphate (NP CS/TPP) containing GA_3 was compared with free GA_3 and with NPs without GA_3. It was verified that the use of nanoencapsulated GA_3 resulted in a more efficient germination response in *D. walteriana* seeds, using smaller doses of the regulator (between 0.75 mg · L^{-1} and 1.0 mg · L^{-1}), mainly with the ALG/CS NPs. Therefore, the use of GA_3 is recommended for *D. dusenii* and *D. walteriana*, and for the latter, nanoparticles containing ALG/CS-GA_3 allow a reduction in the required dose.

Keywords: bromeliads, domestication, gibberellins, nanotechnology, plant growth regulators

INTRODUCTION

Brazil has great floristic diversity, especially in Bromeliaceae, with 1,712 species catalogued (Gouda et al., 2022). Among the states with the highest concentration of species, Bahia (358), Espírito Santo (334), Rio de Janeiro (333) and Minas Gerais (331) stand out, with species occurring mainly in the Atlantic Forest (940) and Cerrado (255) (Reflora, 2023). The production of bromeliads on a commercial scale is a viable and well-explored activity in Brazil, with highlighted use in landscaping projects and for decoration of indoor environments, due to the easy maintenance, hardiness and acclimatisation of these plants to different

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environments (Anacleto and Negrelle, 2013). The species present different colours and leaf shapes, which increases their added value. In this sense, bromeliads of the genus *Dyckia* sp. stand out for the absence of the tank, commonly present in these plants, and for their aesthetically original morphology (Pinangé et al., 2017).

Due to the increased use of bromeliads in Brazilian landscaping, large numbers of plants end up being removed from nature, which is one of the factors contributing to the increase in endangered species (Pereira et al., 2010). Among the *Dyckia* species, for example, *D. walteriana* Leme. falls into the 'critically endangered' category, requiring urgent measures for the conservation of populations (Musegante et al., 2020; Reflora, 2023).

Interest in the propagation of native species has intensified due to the growing concern with environmental problems. However, the knowledge available for the management and analysis of the seeds of most species is still limited, making it difficult to characterise their physical and physiological attributes. It is crucial to understand the behaviour of growth and development factors in native species as this allows the creation of cultivation protocols. These protocols, in turn, make it possible to introduce new species of economic and ornamental value to the market, in addition to contributing to the recovery of already degraded areas (Ribeiro et al., 2012; Borges et al., 2018).

For the germination process to occur uniformly and with the highest possible percentage, some procedures can be adopted in the seeds, such as the application of plant growth regulators, for example, gibberellic acid (GA_3). Gibberellins promote germination through several metabolic state changes in the seed that allow the embryo to re-establish its activity (Hossel et al., 2018).

One way of stimulating germination provided by GA_3 is through the induction of the production of hydrolytic enzymes (such as amylases) in the endosperm, which break down starch and other reserve substances, thus generating soluble organic compounds that provide energy for the germinating embryo (Hossel et al., 2018). There are reports of improvement in germination and overcoming dormancy of seeds of several species with the use of GA_3 (Hossel et al., 2018). Despite this, one of the obstacles to the application of plant growth regulators is that they are easily degraded when exposed to environmental factors, such as light and temperature, resulting in the loss of their activity. In this context, the use of nanotechnology aims to provide nanoscale materials capable of improving stability and activity, in addition to reducing possible environmental impacts (Ashraf et al., 2021). Thus, nanoencapsulation provides advantages such as longer action time, increased biological activity, and reduced concentration required for the desired effect. Another important point is that the material that makes up the particles produces non-toxic metabolites and is easily degraded, hence the option to use natural biodegradable polymers, such as alginate and chitosan (Francisco and García-Estepa, 2018; Pascoli et al., 2018).

Studies associating nanoparticles (NPs) containing plant regulators such as GA_3 are still in infancy and mainly aimed at large crops such as beans (*Phaseolus vulgaris* L.) (Pereira et al., 2017), cucumber (*Cucumis sativus* L.) (Yang et al., 2018) and tomato (*Solanum lycopersicum* var. *cerasiforme*) (Pereira et al., 2019). Therefore, the application of this technology to improve the germination of seeds with low vigour and for use in ornamental species, such as those of the genus *Dyckia,* is innovative. Thus, the current work aimed to evaluate the effect of GA_3 in four species of *Dyckia*. In addition, the potential of nanoencapsulated GA_3 to stimulate seed germination in one of the species responsive to the growth regulator was evaluated.

MATERIALS AND METHODS

Plant material and lot characterisation

The experiment was conducted at the Seed Laboratory of the State University of Londrina (UEL). Ripe fruits of *D. cabrerae* L. B. Smith & Reitz, *D. dusenii* L. B. Smith, *D. pottiorum* Leme., and *D. walteriana* Leme. were collected from mother plants, approximately 90 days after flowering, in October 2020, on the property of collector Dr. Walter Miguel Kranz, located in the municipality of Londrina/PR (23°22′ S and 51°11′ O).

The fruits were allocated in Kraft® (Londrina-PR) paper bags, identified and transported to the laboratory. In a shaded place, they were dried in the open air for 3 days to facilitate the extraction of the seeds, which were stored in Kraft® paper bags, in the refrigerator at 7.5 ± 1.0°C and at a relative humidity (RH) of 26 ± 7%, for 270 days. For the characterisation of the lot, the water content and viability of the seeds were determined by the tetrazolium test, both following the specifications of the Rules for Seed Analysis (BRASIL, 2009). This characterisation occurred after the harvest (10 days) and before the installation of the experiment (after 270 days).

For the determination of water content, 0.2 g of seeds of each species were placed in an oven at 105 ± 3°C for 24 hr. For carrying out the tetrazolium test, four repetitions of 25 seeds each were used. For each species, the seeds were placed in cryovials with a capacity of 2 mL and subsequently completed with distilled water. After 24 hr at 25°C in a germination chamber, the water was removed, and a 1% tetrazolium salt solution was added. The seeds then remained in a germination chamber in the absence of light for 24 hr at 30°C. After this period, the percentage of viable seeds was evaluated with the aid of a magnifying glass. To calculate the percentage of viable seeds, empty seeds were not considered, that is, those without an embryo.

Preparation of formulations

Gibberellic acid (GA_3—90%), sodium alginate (ALG), chitosan (CS) and tripolyphosphate (TPP) were obtained

from Sigma-Aldrich® (São Paulo-SP). The NPs were provided by Professor Dr. Leonardo Fernandes Fraceto from Universidade Estadual Paulista (UNESP/Sorocaba) following the methodology of Pereira et al. (2017).

For the preparation of free GA_3 solutions, the product was weighed and diluted in alcohol (70%), and then distilled water was added to obtain an initial concentration of 50 mg · L^{-1}. Next, this solution was diluted in distilled water to obtain the desired doses (0.25, 0.5, 0.75, 1.0, 2.5 and 5.0 mg · L^{-1}).

The nanoformulations were prepared at an initial concentration of 50 mg · L^{-1}. Alginate/chitosan (ALG/CS) NPs were prepared by the ionotropic pre-gelation method. Initially, with the aid of a peristaltic pump (Miniplus 3, Gilson® (São Paulo-SP)), 3.75 mL of calcium chloride ($CaCl_2$) solution (18 mM) was added to 59 mL of ALG solution (0.063%, pH 4.9) under strong magnetic stirring (500 rpm). During this step, cross-linking of $CaCl_2$ on ALG occurs through ionic interactions, forming a structure called an 'egg box'.

Subsequently, GA_3 was added under stirring until complete dissolution to reach the desired concentration (50 mg · L^{-1}). The ALG/$CaCl_2$/GA_3 solution was kept under agitation, and 12.5 mL of an aqueous solution of chitosan (0.07% pH 4.6), prepared in an aqueous solution containing 5.7% of acetic acid, was added over 90 min, forming a polyelectrolyte complex between polymers. The same process occurred without the presence of the GA_3 regulator to obtain the ALG/CS NPs without the growth regulator.

Chitosan/tripolyphosphate (CS/TPP) NPs were prepared by the gelling method, with modifications. First, 10 mL of a CS solution (0.2%, pH 4.5), prepared in an aqueous solution of 0.6% acetic acid, was kept under vigorous agitation (500 rpm), and GA_3 was added until the final concentration of 50 mg · L^{-1}. After dissolution of GA_3, 6 mL of TPP solution (0.1%, pH 4.5 at 4°C) was added. CS/TPP NPs were also prepared without the presence of the regulator.

The formulations were characterised by size, zeta potential, encapsulation efficiency (quantified by high-performance liquid chromatography—HPLC), the polydispersity index (PDI) and pH. The ALG/CS NPs with and without GA_3 showed an average size of 450 ± 10 nm, PDI of 0.3, zeta potential of –29 ± 0.5 mV, pH of 4.9% and 100% encapsulation efficiency. CS/TPP NPs with and without GA_3 showed an average size of 195 ± 1 nm, PDI of 0.3, zeta potential of +27 ± 3 mV, pH of 4.7 and 90% encapsulation efficiency. Both NPs remained stable for 60 days at room temperature (Pereira et al., 2017).

Treatments

In all experiments, the seeds were sterilised in a 1% sodium hypochlorite solution (1 min), followed by immersion in 70% alcohol (1 min) and subsequently washed with distilled water. They were then soaked in their respective treatments in Erlenmeyer flasks (50 mL), containing 5 mL of the formulation, and shaken for 5 min at room temperature. In the control treatment (0 mg · L^{-1}), the seeds were pre-soaked in distilled water.

In the work with free GA_3, seeds of *D. cabrerae*, *D. dusenii*, *D. pottiorum* and *D. walteriana* were submitted to doses of 0.25, 0.5, 0.75, 1.0, 2.5 and 5 mg · L^{-1} of GA_3. In the experiment with NPs, *D. walteriana* seeds were submitted to the following treatments: NPs of ALG/CS and CS/TPP containing GA_3 and NPs of ALG/CS and CS/TPP without GA_3. The doses used were 0.25, 0.5, 0.75, 1.0, 2.5 and 5.0 mg · L^{-1} of GA_3 and the corresponding dilutions of the NPs without the regulator. Free GA_3 data in this experiment were based on the previous experiment.

Assessments

For each species, tests were carried out for the first and final germination count since there is no recommendation in the Rules for Seed Analysis for the studied species. The 4th day was considered for the first count and the 10th day for the end of the test. The percentage of germination (GERM) and abnormal seedlings (AS), first germination count (FGC), germination speed index (GSI), mean germination time (MGT), seedling length (SL) and seedling dry mass (SDM) were considered. For SL and SDM, the seedling as a whole was considered, that is, with aerial part and root.

The seeds were placed to germinate on blotting paper moistened with distilled water in the amount of 2.5 times the mass of the non-hydrated paper and placed in crystal polystyrene boxes (Gerbox® (DicaLab – Londrina-PR)) with dimensions 11 cm × 11 cm × 3 cm. The Gerbox® boxes were kept in a growth chamber (B.O.D. type) at 25°C for a photoperiod of 16 hr and under an illuminance of 120 µmol · s^{-1} · m^{-2} from fluorescent lamps (BRASIL, 2009).

The GERM was determined considering the normal seedlings, and the AS were also evaluated (BRASIL, 2009), being considered normal those that showed the potential to continue their development and give rise to normal plants when developed under favourable conditions. The FGC was performed at the time of primary root protrusion, which occurred on the 4th day (Nakagawa, 1999). The result was expressed as a percentage.

Concomitantly to the germination test, the count of germinated seeds was performed to establish the GSI obtained through the formula described by Maguire (1962). $GSI = G_1/N_1 + G_2/N_2 + \ldots + G_n/N_n$, in which G_1, G_2 and G_n are the number of normal seedlings, computed in the first, second and last counts, respectively, and N_1, N_2 and N_n are the number of sowing days at the first, second and last counts, respectively. The MGT was obtained through the methodology described by Labouriau (1983) and performed simultaneously with the germination test, the number of germinated seeds being counted daily. This index is calculated by the MT equation $= (G_1T_1 + G_1T_1 + \ldots + G_nT_n)/(G_1 + G_2 + \ldots + G_n)$, where MT is the mean time, in days, required to reach

maximum germination; G_1, G_2 and G_n are the number of germinated seeds at times T_1, T_2 and T_n, respectively.

At the end of 10 days, the GERM and AS were determined, and the result was expressed as percentage, in addition to the SL (mm) through the measurement of normal seedlings and obtained with the aid of a calliper. The SDM (mg) was determined on an analytical scale (precision ±0.0001 g), after keeping the seedlings in paper bags and ovens with forced air circulation at 65°C until they reached constant mass.

Imbibition curve

Aiming to identify the phases of the germination process, the seeds were weighed at intervals of 1 hr (in the first 8 hr) and then every 2 hr until 100 hr had passed from the beginning of the weighing. The seeds were placed on blotting paper moistened with distilled water, in a volume 2.5 times the dry weight of the paper and placed in Gerbox® boxes. In the treatment with GA_3, the seeds were soaked for 5 min at doses of 0.75 mg · L^{-1} for the species *D. cabrerae* and *D. pottiorum* and 5.0 mg · L^{-1} for *D. dusenii* and *D. walteriana*. Seeds without treatment with GA_3 were soaked in distilled water for the same 5 min. Next, the seeds were placed on blotting paper. Before obtaining the mass of the seeds (g), they were superficially dried using germination paper, and the mass was obtained on a semi-analytical scale.

Experimental design and statistical analysis

The experimental design was completely randomised in both stages. The first stage consisted of seven treatments containing different doses (0, 0.25, 0.5, 0.75, 1.0, 2.5 and 5.0 mg · L^{-1}), and the second stage was composed of five treatments (free GA_3, NP ALG/CS/GA_3, NP ALG/CS, NP CS/TPP/GA_3 and NP CS/TPP) with seven doses in each one (0, 0.25, 0.5, 0.75, 1.0, 2.5 and 5.0 mg · L^{-1}). For the germination test of the two stages and imbibition curve, at each dose, four repetitions of 50 seeds were used. For measurements of length and dry mass, 16 seedlings were randomly selected per replication, and the result was expressed as the average of the four seedlings.

The assumptions of normality of errors and homogeneity of variances were tested using Shapiro–Wilk and Bartlett ($p \geq 0.05$) tests, respectively. Then, the data were subjected to analysis of variance at 5% significance. When a significant dose effect was observed, linear or non-linear regression analysis was performed (quadratic, 3- or 4-parameter logistic, segmented, Brain–Cousens logistic model). To obtain the imbibition curves, the data were adjusted using cubic models. To perform the analyses, the AgroR package (Shimizu et al., 2022) of R software was used (R Core Team, 2022).

RESULTS

Lot characterisation

For the characterisation of the seed lot, the values of viability and water content of the seeds are shown in Table 1. The four species studied presented high viability immediately after harvesting, ranging from 78% to 88%, and germination from 71% to 84%. After storage for 270 days in a dry and cold place, there was a reduction in viability to values between 68% and 73% and germination to 36% to 45%. In addition, the water content remained low after storage, ranging from 8.4% to 9.5% after harvest to 7.7% to 8.9% after storage. However, it is noteworthy that a reduction in viability and a significant decrease in germination over time were observed, indicating a possible induction of dormancy caused by the storage period. In addition, pathogens/fungi were not identified in the seeds during the conservation period and in the germination test.

Effect of GA_3 on Dyckia species

Table 2 presents the *p*-value of the analysis of variance of the evaluated variables of bromeliad seeds (*D. cabrerae, D. dusenii, D. pottiorum* and *D. walteriana*) according to the different doses of GA_3. There was a response to GA_3 doses for all species. AS did not respond to GA_3 in any of the species, ranging from 2% to 4%. For *D. cabrerae*, only the SDM variable differed between doses of GA_3. For *D. pottiorum*, there was no response for GERM, MGT, SL and SDM, while *D. walteriana* and *D. dusenii* responded to all variables (except AS).

D. cabrerae showed an increase in SDM up to the estimated dose of 1.2 mg · L^{-1}, obtaining a mass of 4.5 mg at this dose, and from this point on, there was a reduction. In the control, for example, the SDM was 3.1 mg (Figure 1). For *D. dusenii*, all variables, except AS, showed responses under GA_3 doses (Figure 2).

Table 1. Viability by tetrazolium test (%), germination (%) and water content (%) of *Dyckia* spp. after harvest (10 days) and after storage (270 days).

Species	Viability		Germination		Water content	
	10 days	270 days	10 days	270 days	10 days	270 days
Dyckia cabrerae	79	68	71	38	9.2	8.5
Dyckia dusenii	81	71	75	36	9.5	8.9
Dyckia pottiorum	88	69	84	45	8.7	8.1
Dyckia walteriana	78	73	75	37	8.4	7.7

Table 2. *p*-value of the *F* test of the analysis of variance and CV (%) for the variables: FGC (%), GERM (%), AS (%), GSI, MGT (days), SL (mm) and SDM (mg) of seedlings for the species *Dyckia cabrerae*, *D. dusenii*, *D. pottiorum* and *D. walteriana* as a function of doses of GA_3.

	FGC	GERM	AS	GSI	MGT	SL	SDM
			D. cabrerae				
p-value	0.8012	0.8558	0.9081[t]	0.8954	0.7463	0.8915	<0.001
CV (%)	41.14	16.33	50.42	7.35	4.86	5.49	10.10
				D. dusenii			
p-value	0.0037	<0.001	0.9844[t]	<0.001	0.0211	0.0011	<0.001
CV (%)	26.34	11.45	40.2	13.00	5.40	8.65	11.08
				D. pottiorum			
p-value	0.0102	0.3135	0.8692[t]	0.9646	0.0166	0.0403	<0.001
CV (%)	23.44	14.95	37.86	17.23	7.81	16.02	14.80
				D. walteriana			
p-value	<0.001	<0.001	-	<0.001	<0.001	<0.001	<0.001
CV (%)	15.80	6.00	-	5.18	3.16	2.89	5.78

[t]Data transformed to √x.
AS, abnormal seedlings; CV, coefficient of variation; FGC, first germination count; GA_3, gibberellic acid; GERM, germination; GSI, germination speed index; MGI, mean germination time; SDM, seedling dry mass; SL, seedling length.

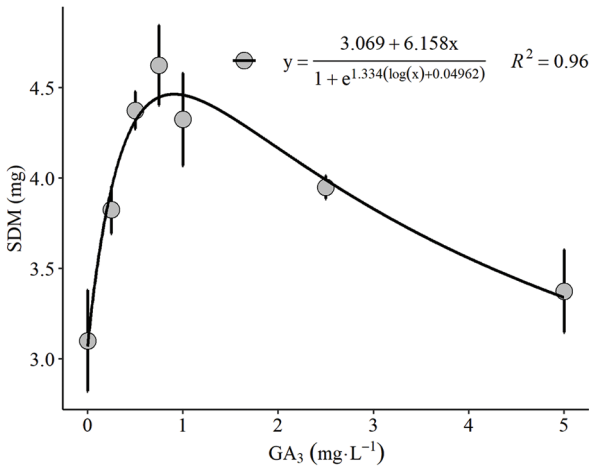

Figure 1. *Dyckia cabrerae* SDM as a function of GA_3 doses. GA_3, gibberellic acid; SDM, seedling dry mass.

For FGC, GERM and SDM, the highest values found were at a dose of 5.0 mg · L^{-1} (21%, 62% and 3.1 g, respectively). In comparison, in the control treatment, the values for the same variables were 10%, 36% and 1.8 g, respectively. MGT was lower at a dose of 5.0 mg · L^{-1}, that is, at this dose, the seeds took 3.00 days for germination.

There was an increase in the GSI with the increased dose of GA_3; however, this index stabilised at higher doses, ranging from 9.53 to 11.08 between doses of 1.0 mg · L^{-1} and 5.0 mg · L^{-1}. A similar response was obtained for SL, so that at doses between 1.0 mg · L^{-1} and 5.0 mg · L^{-1}, seedlings of 7.65 mm and 7.97 mm were obtained. The responses of *D. pottiorum* to GA_3 doses for the variables MGT, FGC, SL and SDM are described in Figure 3.

For FGC, there was an increase in germination up to a dose of 0.8 mg · L^{-1} with 15% germinated seeds. Above this dose, there was a reduction in germination and stabilisation from a dose of 1.0 mg · L^{-1}, ranging from 9% to 11%. For MGT, doses of 0.75 mg · L^{-1} and 1.0 mg · L^{-1} provided a shorter time for seed germination, with 3.20 days and 3.38 days, respectively.

In SL, there was an increase of up to a dose of 1.2 mg · L^{-1}, reaching 7.49 mm, with subsequent reduction in length. For SDM, the estimated dose of 0.7 mg · L^{-1} showed greater mass, with 5.7 mg, and showed a mass reduction for doses above this value. For these variables, in relation to the maximum point obtained, there was an increase of 46% in SL and 56% in SDM compared to the control. *D. walteriana* responded to all variables at different doses of GA_3 (except AS) (Figure 4).

For FGC and GERM, the maximum point obtained was at a dose of 5.0 mg · L^{-1}, with 23% and 64%, respectively. By comparison, the control presented 10% FGC and 35% GERM. In the GSI variable, the highest value was also observed for a dose of 5.0 mg · L^{-1} (with 10.88), as well as the lowest value of MGT (2.98). The same was also observed for the growth variables, with a greater response in SL (8.28 mm) and SDM (3.98 mg) at a dose of 5.0 mg · L^{-1}.

Regarding the imbibition curves, all the species studied showed three-phase water absorption behaviour, with a rapid increase in initial mass, subsequent stabilisation and then a new increase. According to Figure 5, for *D. cabrerae*, phase I lasted 50 hr, and phase III, that is, germination itself, started at 92 hr. On the

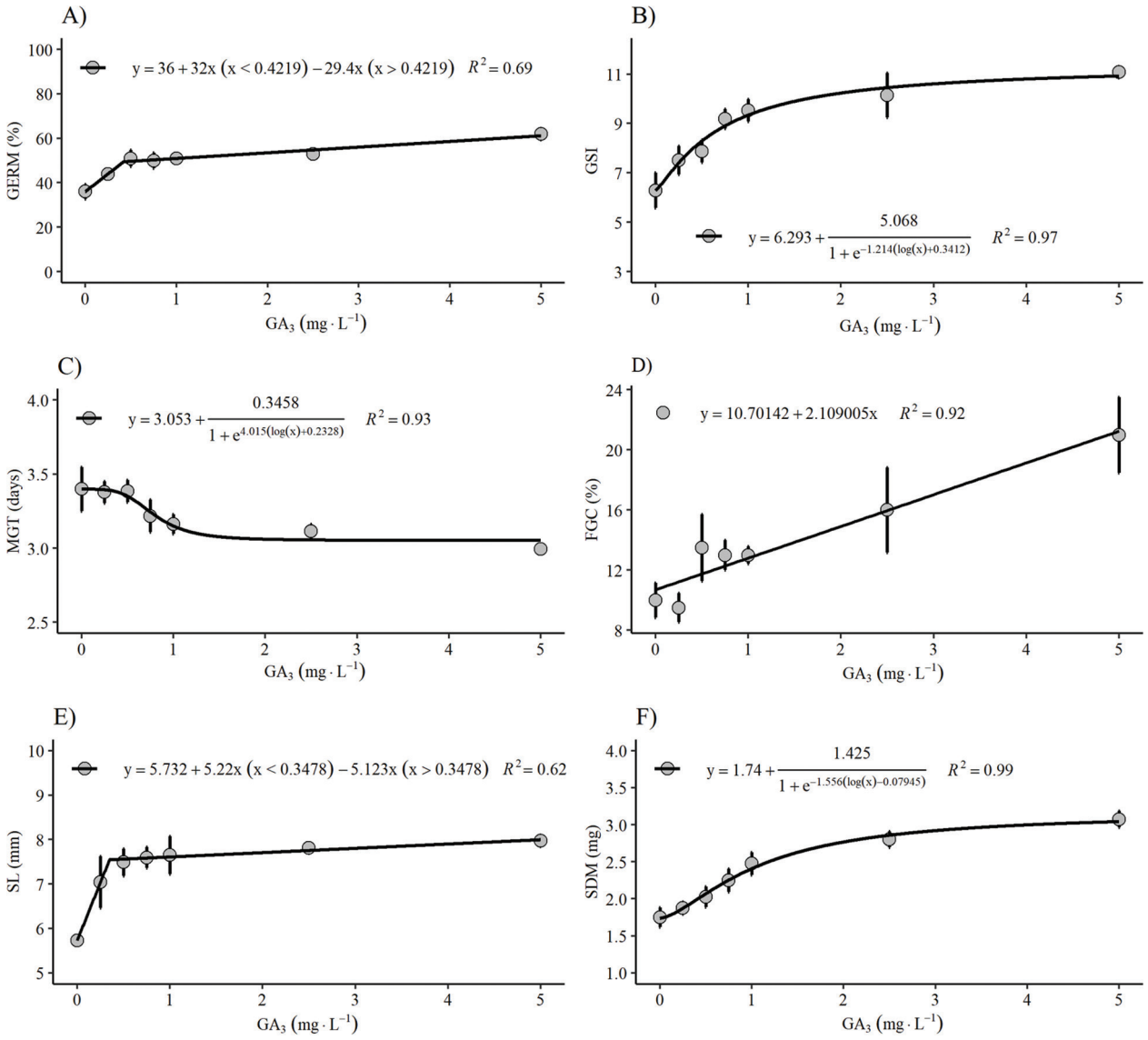

Figure 2. GERM (A), GSI (B), MGT (C), FGC (D), length (SL) (E) and SDM (F) of *Dyckia dusenii* as a function of GA_3 doses. FGC, first germination count; GA_3, gibberellic acid; GERM, germination; GSI, germination speed index; MGT, mean germination time; SDM, seedling dry mass; SL, seedling length.

other hand, when treated with GA_3, phase I lasted 46 hr, and germination started after 90 hr. Phase II lasted 42 hr without treatment with the regulator and 44 hr with the use of the GA_3.

For *D. pottiorum*, phase I lasted 46 hr, and phase III started at 88 hr, while for seeds treated with GA_3, phase I was shorter, lasting 34 hr, and germination started at 90 hr. Phase II lasted 56 hr in treated seeds and 42 hr in seeds not treated with GA_3. For both species, it was possible to observe that even with the GA_3 treatment, there was no great variation in the beginning of germination.

The imbibition curves for *D. dusenii* and *D. walteriana* had similar behaviour to the cited species. However, they were responsive to pre-treatment with GA_3, which reduced the time for the onset of germination, as shown in Figure 6.

For *D. dusenii*, phase I lasted 42 hr, while with GA_3, the time was reduced to 34 hr. Phase II lasted 50 hr for treated seeds, while for untreated seeds, it lasted 48 hr. Germination (phase III) started after 90 hr without GA_3, while with GA_3, it started after 84 hr.

D. walteriana species had phase I lasting 42 hr, which was reduced to 34 hr in seeds treated with GA_3. Phase III, on the other hand, started with 86 hr in the treatment with water only, reducing to 82 hr in the treated seeds.

Effect of nanoencapsulated GA_3 on D. walteriana

After verifying the positive effect of free GA_3 on germination and vegetative growth, mainly in relation to *D. dusenii* and *D. walteriana*, only the latter was chosen for the nanoencapsulated GA_3 tests. The option for *D. walteriana* was due to the beneficial effect of GA_3

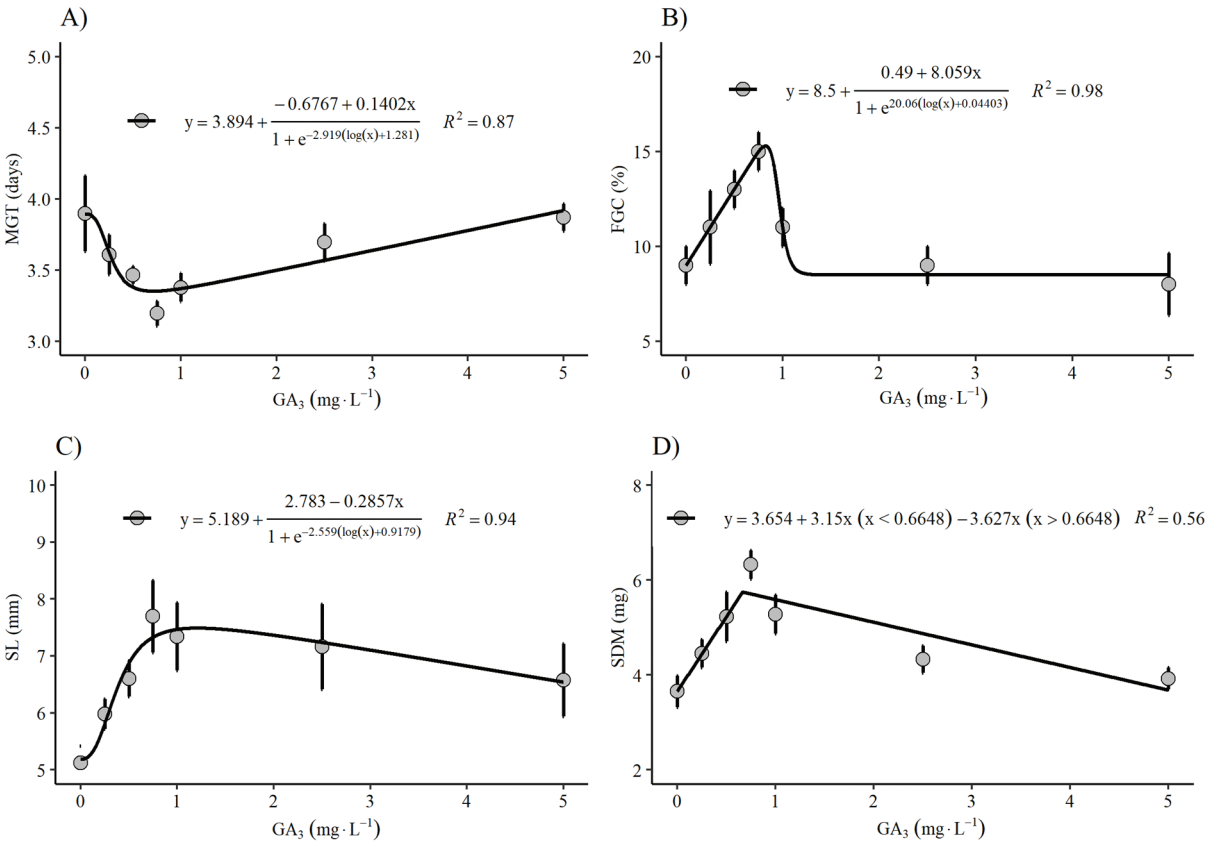

Figure 3. MGT (A), FGC (B), length (SL) (C) and SDM (D) of *Dyckia pottiorum* as a function of GA_3 doses. FGC, first germination count; GA_3, gibberellic acid; MGT, mean germination time; SDM, seedling dry mass; SL, seedling length.

on this species, due to its occurrence in the region where the study was carried out, and due to the higher degree of threat of extinction in which the species is found.

Through analysis of variance, it was possible to observe a significant difference for the various variables according to the treatment. The only exception was BP, which had a variation of 2–3% in all treatments. It was also possible to observe that treatments with only NPs (NP ALG/CS and NP CS/TPP) had no effect on GERM, FGC, MGT, SL and SDM (Table 3).

For FGC, the maximum point obtained for free GA_3 was at a dose of 5 mg · L^{-1} with 23%, while in the treatments with nanoencapsulated GA_3, it was 19% and 18%, in the estimated doses of 1.4 mg · L^{-1} and 0.3 mg · L^{-1} for NP ALG/CS-GA_3 and CS/TPP-GA_3, respectively. In GERM, the highest response was obtained for free GA_3 with 64% at a dose of 5 mg · L^{-1}, and when encapsulated, the maximum response was 60% for NP ALG/CS-GA_3 at an estimated dose of 2.1 mg · L^{-1} and 55% for NP CS/TPP-GA_3 at an estimated dose of 1.7 mg · L^{-1}. The control treatment presented 35% GERM and 10% FGC (Figure 7).

In the GSI and MGT variables, the highest values were also observed for free GA_3 at a dose of 5 mg · L^{-1}, with 10.88 and 2.98, respectively. For treatments with nanoencapsulated GA_3, the estimated dose of 0.5 mg · L^{-1} presented the highest values of GSI and MGT, respectively, of 10.19 and 3.16 for NP ALG/CS-GA_3 and of 9.05 and 3.28 for NP CS/TPP-GA_3. The control treatment presented GSI and MGT, respectively, of 7.05 days and 4.16 days. For MGT, it was also possible to observe a response from empty NPs (NP ALG/CS and CS/TPP), with a maximum response at a dose equivalent to 2.9 mg · L^{-1} for NP ALG/CS with 3.8 days, and 2.7 mg · L^{-1} for NP CS/TPP with 3.8 days (Figure 8).

For SL, when seeds were treated with free GA_3, the dose of 5.0 mg · L^{-1} showed the best response, obtaining average seedlings of 8.28 mm. The same was observed for SDM, with 3.98 mg for the same dose. For NP ALG/CS-GA_3, the estimated optimum dose was 0.3 mg · L^{-1} with 8.04 mm of SL and 3.68 mg of SDM. For NP CS/TPP-GA_3, the estimated dose of 0.2 mg L^{-1} had the best response for SL with 7.78 mm, and the estimated dose of 0.4 mg · L^{-1} presented higher SDM with 3.45 mg. It was also possible to observe an empty response for NP CS/TPP for SL, with 7.26 mm at the equivalent dose of 2.0 mg · L^{-1}. The control treatment presented a mean SL of 6.61 mm and an SDM of 2.15 mg (Figure 9).

DISCUSSION

Lot characterisation

Dyckia seeds are considered orthodox, meaning that they are able to tolerate low humidity levels and storage

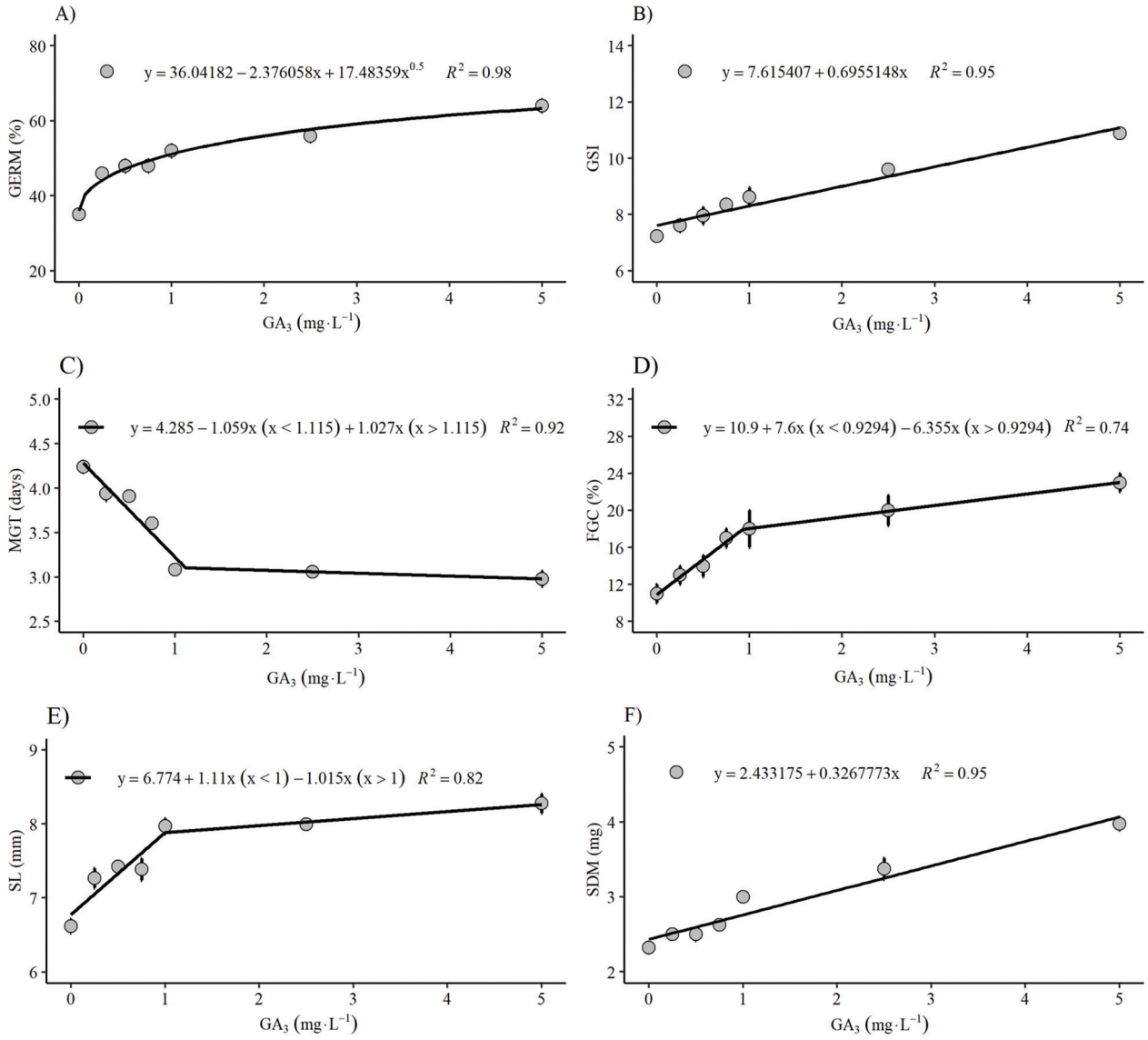

Figure 4. GERM (A), GSI (B), MGT (C), FGC (D), length (SL) (E) and SDM (F) of *Dyckia walteriana* as a function of GA_3 doses. FGC, first germination count; GA_3, gibberellic acid; GERM, germination; GSI, germination speed index; MGT, mean germination time; SDM, seedling dry mass; SL, seedling length.

at cool temperatures. Thus, it is essential to maintain adequate conditions to ensure seed viability for a long period (Zucchi et al., 2018). In addition, the maximum germination potential of seeds is influenced by factors such as the origin of the seeds and environmental conditions during the maturation, harvesting and drying process. Therefore, it is important to understand the physiological behaviour and the ideal storage conditions for the conservation of seed viability (Zucchi et al., 2018; Fior et al., 2020).

In addition to the storage conditions for seeds of the species under study being recommended for seeds with orthodox behaviour (cold and dry), the ideal period for storing and maintaining seed viability is directly related to the water content in which the seeds are conserved. In this sense, the water content, despite being at an adequate level for the studied species (7%–9%), is not applicable to all with the same effectiveness since each one has a tolerable limit regarding desiccation (Rajanaidu and Ainul, 2013).

Even under ideal storage conditions, there was a loss of viability of these seeds since with aging, damage to the membranes occurs and the enzymes lose their catalytic activity (Oliveira et al., 2011). In addition, according to the observed results, the seeds may have entered a state of dormancy after storage. This can also be explained by the tetrazolium test, where despite the low germination after storage, the seeds presented high viability (71–84%). A favourable point of the tetrazolium test is that its results do not suffer interference from conditions that usually interfere in germination analysis, such as the incidence of microorganisms; in addition, this test also serves to clarify factors not explained by the germination test,

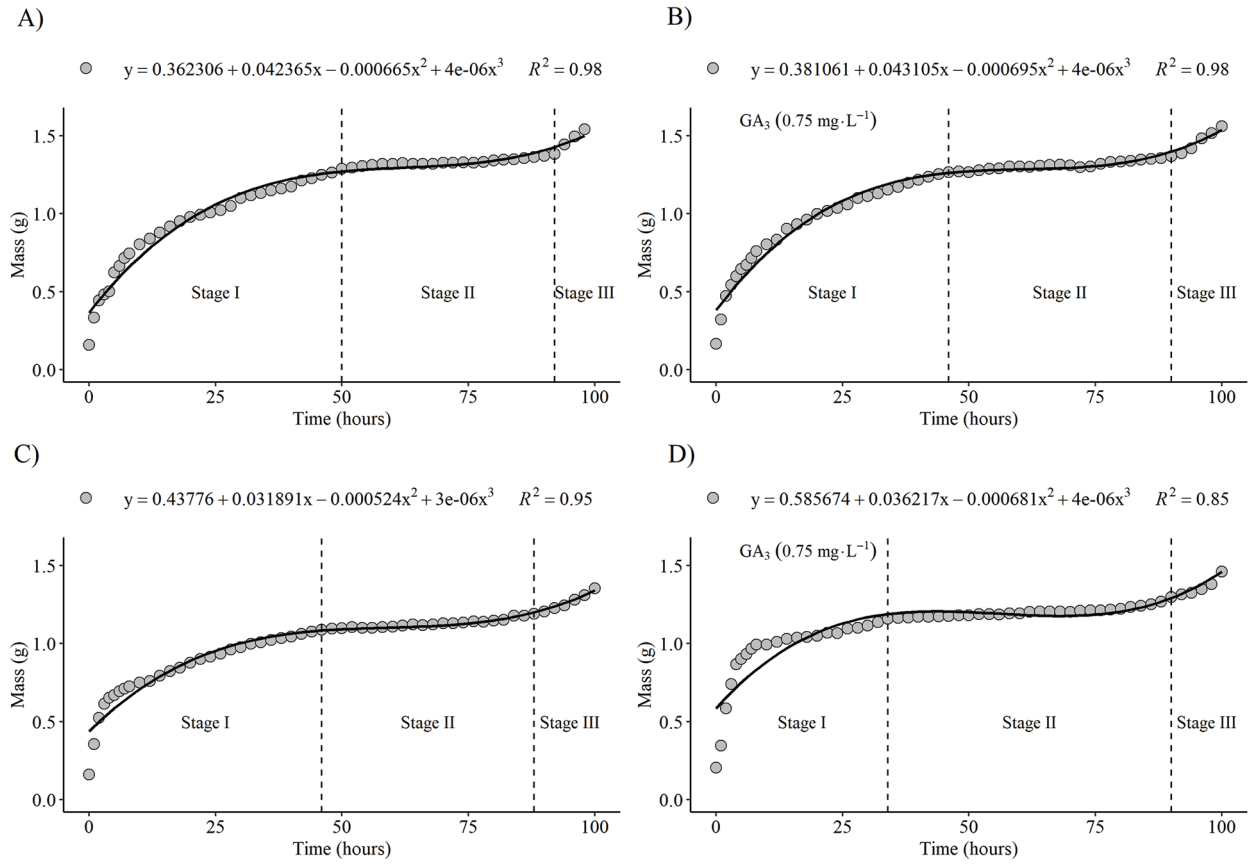

Figure 5. Curve of water absorption by the seeds of *Dyckia cabrerae* without (A) and with GA_3 (B) and *D. pottiorum* without (C) and with GA_3 (D) at a dose of 0.75 mg · L^{-1}. GA_3, gibberellic acid.

in this case, relating to dormancy (Carvalho et al., 2013).

Another associated factor is that most *Dyckia* species present high germination at higher temperatures, significantly reducing germination at lower temperatures; thus, the induction of dormancy under these storage conditions (cold) could have occurred. Dormancy can be characterised as a failure of germination even under apparently favourable conditions for germination. In this case, the dormancy can be divided into primary or secondary. Primary dormancy occurs during the seed maturation phase, that is, it is dispersed already in a dormant state (Silva et al., 2018).

In the case of the seeds under study, this primary dormancy was not observed, so it can thus be classified as secondary dormancy. All the processes involved in this type of dormancy are not yet well elucidated in the literature, and it is recognised that seeds with secondary dormancy germinate normally, but when exposed to unfavourable environmental factors, they are induced to a state of dormancy (Silva et al., 2018). Among the possibilities of dormancy, the physiological possibility occurs through the interaction between inhibitors and germination promoters and is generally overcome through the addition of plant growth regulators, such as GA_3, which was observed in this study (Rego et al., 2018).

Effect of GA_3 on Dyckia species

D. dusenii and *D. walteriana* showed stimulation in the germination process. In this sense, gibberellins (GAs) have the ability to promote seed germination. Thus, a high level of GAs and low level of ABA is a favourable condition for seed germination (Tuan et al., 2018; Zhong et al., 2021). As germination progresses, seed reserves are gradually degraded, providing energy and metabolites for germination and seedling establishment (Xiong et al., 2021).

This GA-induced degradation of seed reserves occurs through the production of hydrolases, which are responsible for weakening the tissue surrounding the embryo (Bocatto and Forti, 2019). Hydrolases, such as amylases, react with the reserves stored in the embryo, breaking down starch and other substances, allowing the resumption of growth of the embryonic axis. With this, simple sugars, amino acids and nucleic acids are formed that stimulate cell elongation, causing the radicle to break the seed coat, accelerating and standardising germination (Paixão et al., 2021).

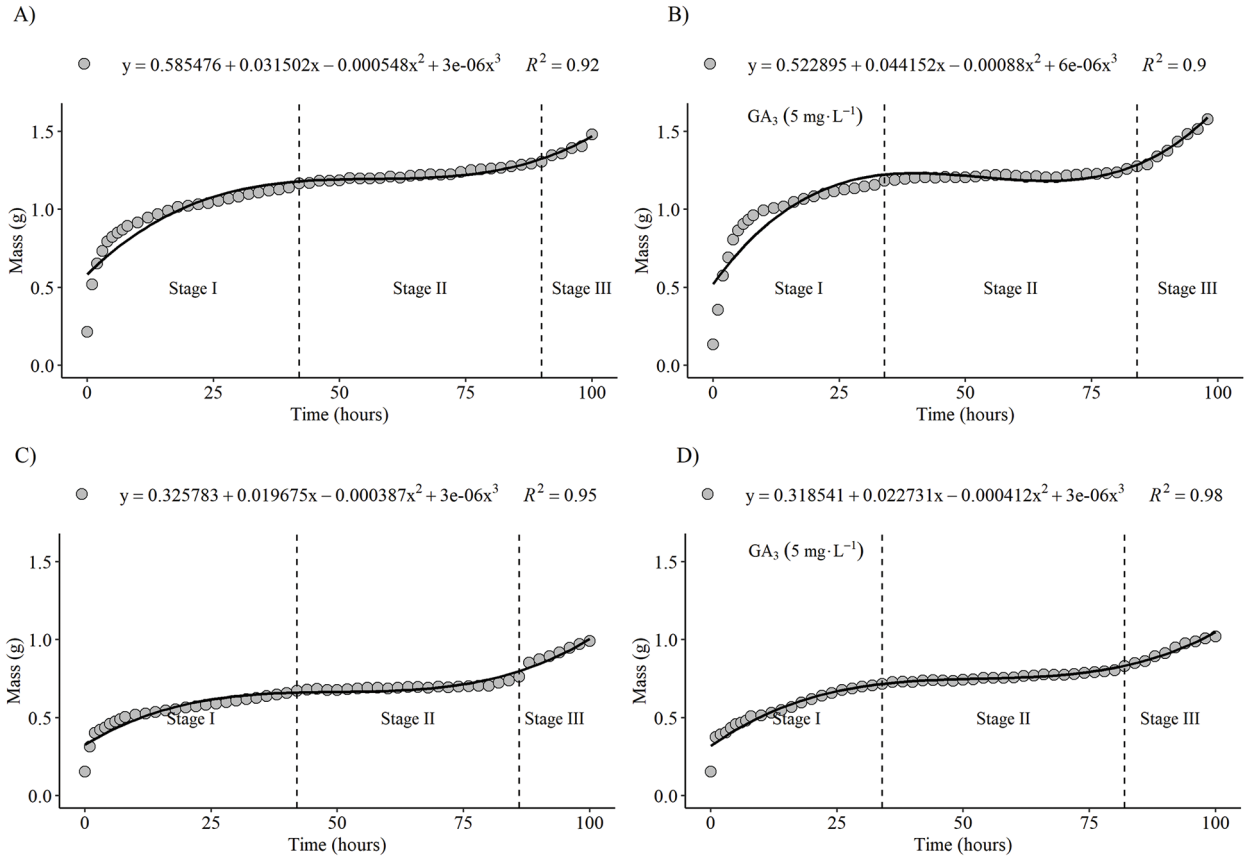

Figure 6. Water absorption curve by *Dyckia dusenii* seeds without (A) and with GA_3 (B) and *D. walteriana* without (C) and with GA_3 (D) at a dose of 5.0 mg · L^{-1}. GA_3, gibberellic acid.

Table 3. *p*-value analysis of variance and CV (%) for the variables: FGC (%), GERM (%), AS (%), GSI, MGT (days), SL (mm) and SDM (mg) of seedlings for treatments NP ALG/CS-GA_3, NP CS/TPP- GA_3, NP ALG/CS and NP CS/TPP at different doses.

	FGC	GERM	AS	GSI	MGT	SL	SDM
				NP ALG/CS-GA_3			
p-value	<0.001	<0.001	-	<0.001	<0.001	<0.001	<0.001
CV (%)	14.56	5.41	-	3.41	3.65	2.35	7.09
Mean	-	-	3.14	-	-	-	-
				NP CS/TPP-GA_3			
p-value	<0.001	<0.001	-	<0.001	<0.001	<0.001	<0.001
CV (%)	14.97	7.24	-	4.23	3.27	1.97	6.44
Mean	-	-	2.29	-	-	-	-
				NP ALG/CS			
p-value	0.414	0.064	-	<0.001	0.024	0.053	0.474
CV (%)	22.68	10.74	-	6.08	3.20	4.70	11.18
Mean	11.0	42.0	2.71	7.45	-	7.06	7.09
				NP CS/TPP			
p-value	0.669	0.057	-	<0.001	0.006	0.003	0.406
CV (%)	22.09	11.18	-	3.49	2.56	2.72	8.28
Mean	11.0	38.0	3.14	7.06	-	-	2.24

ALG, sodium alginate; ALG/CS, alginate/chitosan; AS, abnormal seedlings; CS, chitosan; CV, coefficient of variation; FGC, first germination count; GA_3, gibberellic acid; GERM, germination; GSI, germination speed index; MGT, mean germination time; NP, nanoparticle; SDM, seedling dry mass; SL, seedling length; TPP, sodium tripolyphosphate.

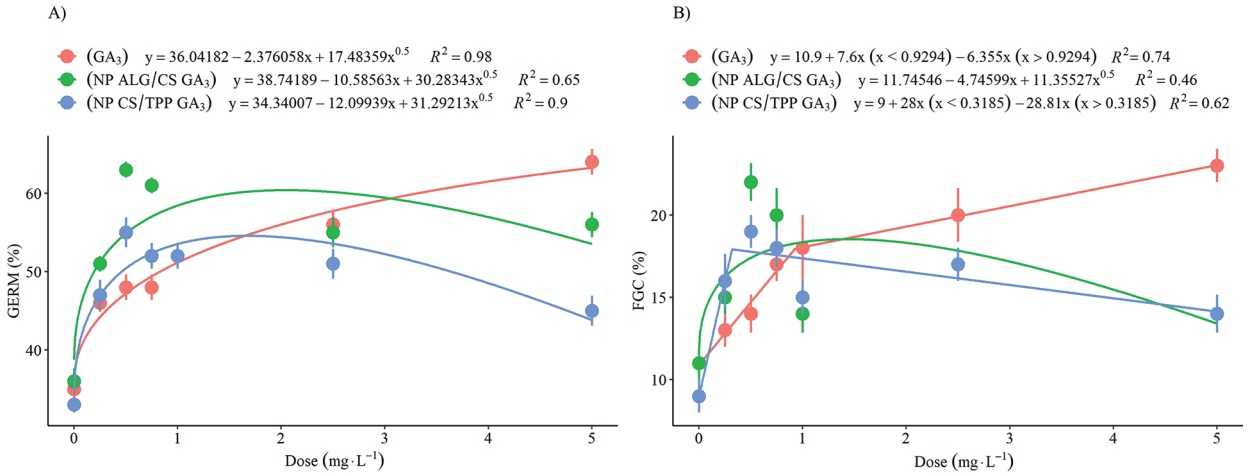

Figure 7. GERM (A) and FGC (B) of *Dyckia walteriana* seeds as a function of different doses and types of gibberellic acid formulation (GA_3). ALG, sodium alginate; CS, chitosan; FGC, first germination count; GA_3 gibberellic acid; GERM, Germination; NP, nanoparticle; TPP, sodium tripolyphosphate.

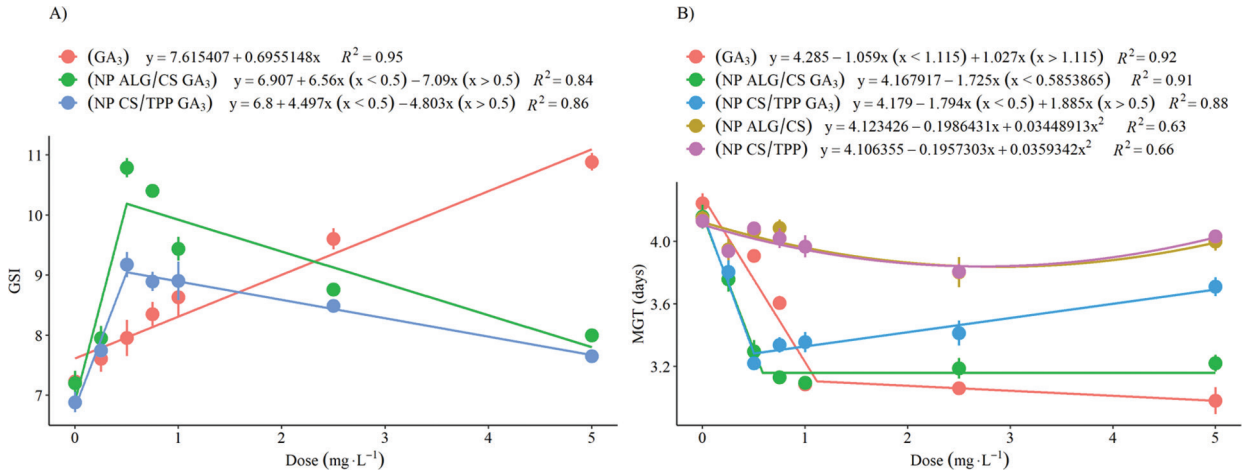

Figure 8. GSI (A) and MGT (B) of *Dyckia walteriana* seeds as a function of different doses and types of gibberellic acid formulation (GA_3). ALG, sodium alginate; CS, chitosan; GA_3 gibberellic acid; GSI, germination speed index; MGT, mean germination time; NP, nanoparticle; TPP, sodium tripolyphosphate.

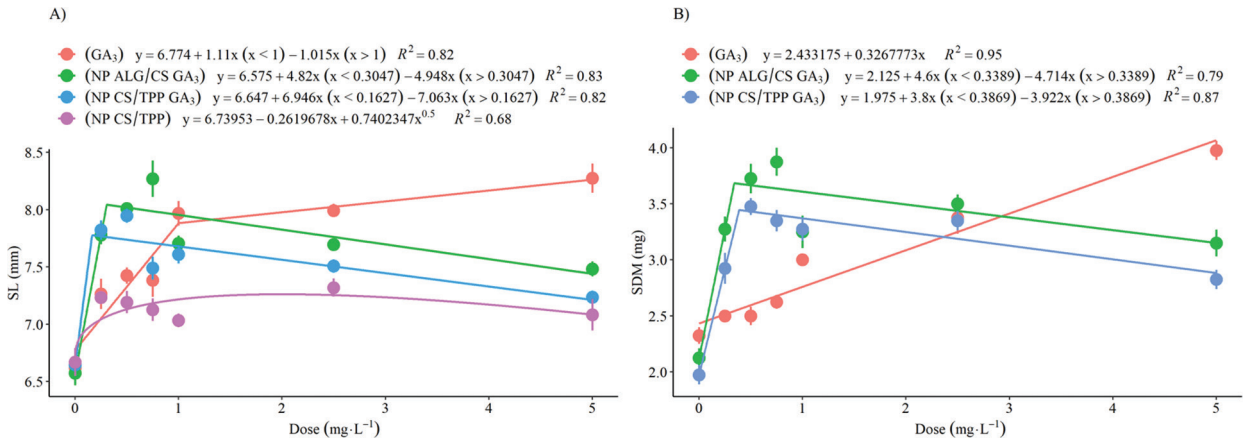

Figure 9. Length (SL) (A) and dry mass (SDM) (B) of *Dyckia walteriana* seedlings as a function of different doses and types of gibberellic acid formulation (GA_3). ALG, sodium alginate; CS, chitosan; GA_3 gibberellic acid; NP, nanoparticle; SDM, seedling dry mass; SL, seedling length; TPP, sodium tripolyphosphate.

It was observed in *D. cabrerae* and *D. pottiorum* that GA_3 did not improve germination parameters, as also observed by Pompeli (2006) in studies with seeds of *Dyckia encholirioides* (Gaudichaud) Mez., verifying that the application of GA_3 did not result in the germinal stimulus. The fact that these species respond in growth, but not in germination, is an indication that GA_3 is not acting in the embryo, either because it cannot get in contact with it or because it is inactivated. However, after radicle protrusion, there is contact with the regulator, and it starts to have the expected effect. It should also be taken into account that environmental abiotic parameters such as light and temperature can affect aspects of plant development, in this case, altering the concentrations of gibberellins (GAs) and/or altering the plant's ability to respond to this plant growth regulator (Kashiwaqui et al., 2019).

The regulation of endogenous levels of GAs in seeds, as well as in the plant, occurs through several processes. Internally, the so-called active GAs undergo the process of conjugation, that is, sugars such as glucose unite with the GAs, making them glycosylated and thus reversibly inactive. GAs can also be irreversibly inactivated by enzymes. These mechanisms control and contribute to the balance in the levels of GAs; in addition, studies demonstrate several inhibitors that act in the process of biosynthesis of GAs (Kashiwaqui et al., 2019).

Regarding the improvement in plant growth variables, that is, length (SL) and mass (SDM), all species were stimulated by GA_3. Gibberellins promote these responses through several factors, such as the orientation of microtubules towards the axis of cell growth; thus, cells increase only towards the axis of growth (Chaudhary et al., 2019; Purba et al., 2021).

GAs stimulate cell division, especially meristematic cells, and affect cell wall plasticity, favouring growth. Furthermore, GAs have the potential to stimulate active plant growth, such as stem and leaf elongation. GAs also interact with other hormones, such as cytokinins and auxins, enhancing these effects (Gupta and Chakrabarty, 2013; Chaudhary et al., 2019; Purba et al., 2021).

Research shows that the use of GA_3 has an effect at different stages of plant development. Treatment with 100 μM of GA_3 promoted greater height and a greater number of flower buds in jewelweed (*Impatiens hawkeri* W. Bull) (Verdolin et al., 2021). Lakshmaiah et al. (2019) obtained better flowering quality and delayed yellowing of lisianthus leaves (*Eustoma grandiflorum* (Raf.) Shinners) with the application of 150 ppm of GA_3.

The pre-germination treatment of beton seeds (*Rhaphiodon echinus* Shauer.) with GA_3 at concentrations of 0.5 and 1.0% increased the germination process and decreased the average germination time (Souza et al., 2018). The treatment of imperial bromeliad seeds *Alcantarea imperialis* (Carriere) Harms (Bromeliaceae) with 5.0 mg · L^{-1} GA_3 promoted greater germinability in relation to the other treatments (Bonin et al., 2010).

The imbibition curve data indicate a triphasic pattern of water absorption by the seeds. It is known that the speed of occurrence of this process depends on the characteristics of the seeds of each species, such as the chemical composition and permeability of the seed coat (Albuquerque et al., 2009). Phase I results in rapid absorption of water and is characterised by the beginning of the modification of the reserves present in the seeds to guarantee energy and essential nutrients for the growth of the embryo (Araújo et al., 2018).

In phase II, water absorption decreases and stabilisation occurs, being a phase also known as stationary. Another characteristic of this phase is the reactivation and initiation of cellular metabolic processes, with the expansion of the embryo and increased activity of enzymes used for embryo development. Finally, in phase III, there is again an increase in water absorption by the seed due to seedling growth and cell reorganisation. The striking characteristic of this phase is that germination becomes visible, that is, the radicle protrudes (Araújo et al., 2018).

Morphological studies carried out by Duarte et al. (2009) in *D. goehrinii* Gross & Rauh suggest the formation of chalaza, which appears as a dark spot on the seed coat, allowing the nucella to become highly vascularised. This characteristic observed in this species may help explain why water absorption occurs so quickly in *Dyckia* species; however, studies are needed for this confirmation.

According to the imbibition curve, none of the species of *Dyckia* (*D. cabrerae*, *D. dusenii*, *D. pottiorum* and *D. walteriana*) presented seeds with dormancy, at least the integument. Tegumentary dormancy, considered a mechanical restriction, is discarded due to the fact that there was no resistance to the flow of water into the seeds, that is, the seeds were able to absorb water. Thus, the possibility is that the seeds had entered secondary or physiological dormancy (Rego et al., 2018), which is also evidenced by the positive response generated by GA_3.

It is also noted that GA_3 acted on the embryo of the most responsive species (*D. dusenii* and *D. walteriana*) by accelerating the germination process through reducing the imbibition phases. On the other hand, in *D. cabrerae* and *D. pottiorum* seeds, GA_3 only reduced phase I and lengthened phase II, with no effect on speed and percentage of germination. These species were responsive to GA_3 in parameters related to the seedlings, suggesting that some internal mechanism of the embryo inactivated GA_3 and, after germination, the radicles of the seedlings, when in direct contact with the GA_3, underwent modifications, with an increase in growth and mass, thus responding to the regulator (Kashiwaqui et al., 2019).

Effect of nanoencapsulated GA_3 on D. walteriana

The purpose of application through NPs is to provide slow and sustained release of the active substance, in addition to protecting it against degradation

(Pereira et al., 2017). One of the advantages of using biodegradable polymeric systems is that they can be used in the metabolism of living organisms. Furthermore, in this study, none of the NP systems used showed phytotoxic effects during the germination phases, corroborating the results found in the literature (Pereira et al., 2019).

It was observed that the responses for freely applied and nanoencapsulated GA_3 had different maximum response points, demonstrating that the form of application provided different biological effects. The greatest effects on variables using NPs were observed at lower doses (0.75mg · L^{-1} and 1.0 mg · L^{-1}), while the greatest effects of GA_3 in the free form occurred at the highest doses (2.5 mg · L^{-1} and 5.0 mg · L^{-1}). This can be explained by the fact that free GA_3 had direct contact with the seeds, being released faster than nanoencapsulated GA_3, which provided a slower release. In addition, some NPs remain adhered to the surface of the seed, maintaining the supply of GA_3 even after the soaking process. Furthermore, NPs, mainly CS/ALG due to their negative charge, are more likely to enter the seeds, making it impossible for them to become adsorbed by the cell wall (Pereira et al., 2019).

The CS present in NPs is easily absorbed, prolonging the contact time and facilitating the absorption of bioactive molecules, in this case GA_3. The good acceptability and absorption of NPs are also reported due to the association with the surface of the plant, or seed, in this case, due to the presence of carboxyl, hydroxyl, amide and phosphate groups that provide potential sites for binding with CS, which with its cationic properties, manages to adsorb on the surface, prolonging the contact of the nanoparticle with the plant (Li et al., 2019).

In the study in question, there was no better response with the use of nanoencapsulated GA_3, with emphasis on the dose reduction when supplied this way, probably due to better delivery of GA_3 to the plants. The fact that there was no difference in the two forms of release can be explained by the fact that the study experiment was carried out in a fully controlled environment (temperature and humidity), thus not affecting the degradation and bioavailability of GA_3.

In studies using different nanocarrier systems containing GA_3 through alginate/chitosan (ALG/CS) and CS/TPP, it was demonstrated that the treatment of seeds with ALG/CS-GA_3 was more effective and provided an increase in root development, leaf area and photosynthetic pigments (chlorophylls and carotenoids) of bean plants (*P. vulgaris* L.) (Pereira et al., 2017).

The same result was also found in the present study, in which NPs containing ALG/CS showed a better response than CS/TPP, which was also observed by Pereira et al. (2019), in which tomato seeds (*S. lycopersicum* var. Cerasiforme) treated with the different systems, showed better responses to the NPs of ALG/CS-GA_3, which provided an increase in the dry mass of shoots and roots and photosynthetic pigments, while the CS/TPP-GA_3 formulation showed relatively low biological activity during the initial growth of the plants.

The two NP systems have different GA_3 release mechanisms, causing different responses. Among the NP characteristics, the zeta potentials of the studied formulations differ, being negative for ALG/CS and positive for CS/TPP. Studies show that the zeta potential participates in the plant–NP interaction. NPs with positive zeta potential, such as CS/TPP, have a strong interaction with the negative groups of the plant cell wall, with low cell internalisation and a tendency to accumulate on the cell surface. On the other hand, NPs with negative zeta potential, such as those of ALG/CS, manage to be rapidly distributed and internalised in cells (Zhu et al., 2012).

The effect of NPs containing only ALG/CS or CS/TPP was observed for some variables. Of the compounds used in the formulation of NPs, the ability of CS to increase the response in plants depending on the species and concentration has already demonstrated in several studies, being mainly associated with plant defence responses to different biotic and abiotic stresses (Malerba and Cerana, 2016; Odat et al., 2021).

Chitosan was able to improve seed germination and initial growth of wheat seedlings (*Triticum aestivum* L.) (Peykani and Sepehr, 2018). It was observed that different concentrations of CS resulted in an increase in hypocotyl and radicle length and dry mass in relation to control vetch seeds *Vicia sativa* L. (Odat et al., 2021).

The present study shows the importance of physiological knowledge of seeds after maturation and during storage and that seeds of *Dyckia* species, with the aid of plant growth regulators, return to good germinability even after a long storage period and a reduction in germination potential. In addition, the findings serve as an information base for more detailed studies regarding the acceptance of NPs by the seed and the use of nanotechnology in several areas. These results are promising in the sense of bringing information and possibilities for the cultivation of this group of predominantly native plants and inserting them in the ornamental plant market.

CONCLUSIONS

For *D. dusenii*, a dose of 5.0 mg · L^{-1} is recommended, and for *D. walteriana*, it is between 4.4 mg · L^{-1} and 5.0 mg · L^{-1} of GA_3. The species *D. cabrerae* and *D. pottiorum* responded only to vegetative growth, GA_3 not being recommended for germination.

When nanoencapsulated, GA_3 resulted in greater responses at lower doses, proving the controlled release of the regulator, with greater responses for NPs containing ALG/CS. The dose of 5.0 mg · L^{-1} of free GA_3 and between 0.75 mg · L^{-1} and 1.0 mg · L^{-1} for nanoencapsulated containing ALG/CS-GA_3 are recommended for germination of *D. walteriana*.

FUNDING

The authors would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for granting scholarships to the first and seventh authors (n° 88882.448347/2019-01 and n° 88881.191767/2018-01, respectively) and the National Council for Scientific and Technological Development (CNPq) for funding support to the fifth, sixth and seventh authors (n° 301684/2017-0, n° 311034/2020-9 and n° 308439/2021-0, respectively) and also the São Paulo State Research Support Foundation (FAPESP) for financial support to the seventh author (2017/21004-5 and 21/10639-5).

AUTHOR CONTRIBUTIONS

J.C.B.P and H.C.O. – conceptualisation. J.C.B.P., H.R.G. and G.D.S. – methodology. H.C.O., J.C.B.P., R.T.F., L.F.F. and A.E.S.P. – validation. J.C.B.P., H.C.O., G.D.S. and K.A.M.M. – investigation. G.D.S. – data curation. J.C.B.P., H.C.O., G.D.S. and K.A.M.M. – writing – original draft preparation. H.C.O., R.T.F., L.F.F. and A.E.S.P. – writing – review and editing. H.C.O. – supervision. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Albuquerque, K. S., Guimarães, R. M., Almeida, I. F., and Clemente, A. C. S. (2009). Alterações fisiológicas e bioquímicas durante a embebição de sementes de sucupira-preta. *Revista Brasileira de Sementes*, *31*(1), 12–19, http://dx.doi.org/10.1590/S0101-31222009000100028.

Anacleto, A., and Negrelle, R. R. B. (2013). Produção de bromélias no Estado do Paraná, Brasil. *Revista Brasileira de Horticultura Ornamental*, *19*(2), 121–136, https://doi.org/10.14295/rbho.v19i2.421.

Araújo, R. F., Abud, H. F., Pinto, C. M. F., Araújo, E. F., and Leal, C. A. M. (2018). Curva de embebição de sementes de pimentas biquinho e malagueta sob diferentes temperaturas. *Revista Brasileira de Agropecuária Sustentável*, *8*(3), 51–56.

Asharaf, S. A., Siddiqui, A. J., Elkhalifa, A. E. O., Khan, M. I., Patel, M., Alreshidi, M., Moin, A., Singh, R., Snoussi, M., and Adnan, M. (2021). Innovations in nanoscience for the sustainable development of food and agriculture with implications on health and environment. *Science of the Total Environment*, *768*(10), 144990, https://doi.org/10.1016/j.scitotenv.2021.144990.

Bocatto, S. J., and Forti, V. A. (2019). Métodos para promover a superação de dormência em sementes de Urucum. *Scientia Agraria Paranaensis*, *18*(3), 226–231, http://dx.doi.org/10.18188/sap.v18i3.21546.

Bonin, M. P., Moraes, C. P., Martini, G. A., Benedito, P. V., and Souza-Leal, T. (2010). Avaliação dos tratamentos pré-germinativos em diferentes concentrações de GA_3 na germinação de *Alcantarea imperialis* (Vell.) Harms. *Scientia Plena*, *6*(5), 1–4.

Borges, R. P., Nogueira, L. T., Cecchin, D., Pereira, C. R., and Hunter, C. M. (2018). Germinação de sementes de tomate submetidas a diferentes concentrações de cloreto de sódio e substratos. *Enciclopédia Biosfera*, *15*(28), 571–577, http://dx.doi.org/10.18677/EnciBio2018B49.

Brasil. (2009). Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes (399 p). Brasília: Mapa/ACS.

Carvalho, T. C., Krzyzanowski, F. C., Ohlson, O. C., and Panobianco, M. (2013). Tetrazolium test adjustment for wheat seeds. *Journal of Seed Science*, *35*(3), 361–367.

Chaudhary, A., Adhikari, B. B., and Shrestha, J. (2019). Effect of weed species on drought tolerant rice genotypes at Sundarbazar, Lamjung, Nepal. *Open Agriculture*, *4*(1), 65–78, https://doi.org/10.1515/opag-2019-0006.

Duarte, E. F., Carneiro, I. F., and Rezende, M. H. (2009). Morfologia de frutos e sementes e desenvolvimento pós-seminal de *Dyckia goehringii* Gross & Rauh (Bromeliaceae). *Journal of Neotropical Biology*, *6*(1), 1–12, https://doi.org/10.5216/rbn.v6i1.12626.

Fior, C. S., Campos, S. S., and Schwarz, S. F. (2020). Tolerância à dessecação e armazenamento em temperatura sub-zero de sementes de *Butia odorata* (Barb. Rodr.) Noblick. *Iheringia, Série Botânica*, Porto Alegre, *75*, e2020019, https://doi.org/10.21826/2446-82312020v75e2020019.

Francisco, E. V., and García-Estepa, R. M. (2018). Nanotechnology in the agrofood industry. *Journal of Food Engineering*, *238*(1), 1–11, https://doi.org/10.1016/j.jfoodeng.2018.05.024.

Gouda, E. J., Butcher, D., and Gouda, K. (2022). Encyclopaedia of Bromeliads, version 4. Retrieved from http://bromeliad.nl/encyclopedia/.

Gupta, R., and Chakrabarty, S. K. (2013). Gibberellic acid in plant. *Plant Signaling & Behavior*, *8*(9), e25504, https://doi.org/10.4161/psb.25504.

Hossel, C., Hossel, J. S. A. O., Wagner Júnior, A., Alegretti, A. L., and Dallago, A. (2018). Temperaturas e giberelina na germinação de sementes de *Passiflora caerulea*. *Pesquisa Aplicada & Agrotecnologia*, *11*(1), 93–98, http://dx.doi.org/10.5935/PAeT.V11.N1.11.

Kashiwaqui, M. M., Costa, B. P., Rego, C. A. R. M., Caetano, J. H. S., Sampaio, M. C., Guimarães, V. F., and Da Costa, A. C. T. (2019). Regulators plant: A brief review on gibberellin. *Revista Sodebras*, *14*(166), 11–16, https://doi.org/10.29367/issn.1809-3957.14.2019.166.11.

Labouriau, L. G. (1983). *A germinação das sementes* (173 p). Washington, Estados Unidos: Secretaria Geral da Organização dos Estados Americanos.

Lakshmaiah, K., Subramanian, S., Ganga, M., and Jeyakumar, P. (2019). Optimization of pinching and GA_3 application to improve growth and flowering of Lisianthus (*Eustoma grandiflorum*). *Journal of Pharmacognosy and Phytochemistry*, *8*(6), 614–616.

Li, R., He, J., Xie, H., Wang, W., Bose, S. K., Sun, Y., Hu, J., and Yin, H. (2019). Effects of chitosan nanoparticles on seed germination and seedling growth of wheat (*Triticum aestivum* L.). *International Journal of Biological Macromolecules*, *126*(1), 91–100, https://doi.org/10.1016/j.ijbiomac.2018.12.118.

Maguire, J. D. (1962). Speed of germination-aid in selection and evaluation for seedling emergence and vigor. *Crop Science*, *2*, 176–177, https://doi.org/10.2135/cropsci1962.0011183X000200020033x.

Malerba, M., and Cerana, R. (2016). Chitosan effects on plant systems. *International Journal of Molecular Sciences*, *17*(7), 996, https://doi.org/10.3390/ijms17070996.

Musegante, J. V. R., Ribeiro, J. E. L. S., Ferraz, J. R., Oliveira, P. O., Bianchini, E., and Medri, C. (2020). Geographical distribution of *Dyckia walteriana* (Bromeliaceae), a recently described and endangered species endemic to the Serra Geral formation, southern Brazil. *Phytotaxa*, *438*(4), 263–275, https://doi.org/10.11646/phytotaxa.438.4.5.

Nakagawa, J. (1999). Testes de vigor baseados no desempenho das plântulas. In F. C. Krzyzanowski, R. D. Vieira, and J. B. França Neto (Ed), *Vigor de Sementes: Conceitos e Testes* (pp. 1–24). Londrina: ABRATES.

Odat, N. A., Tawaha, A. R. M. A., Hasan, M., Imran, K., Amanullah, D., Tawaha, A. R. M., Thangadurai, D., Sangeetha, J., Rauf, A., Khalid, S., Saranraj, P., Al-Taey, D. K. A., Safari, Z. S., Zahid, N. A., Qazizadah, A. Z., and Sirajuddin, S. N. (2021). Seed priming with chitosan alleviates salinity stress by improving germination and early growth parameters in common vetch (*Vicia sativa*). *Conference Series Earth and Environmental Science*, *788*(1), 012059, https://doi.org/10.1088/1755-1315/788/1/012059.

Oliveira, J. A., Silva, T. T. A., Pinho, E. V. R. V., and Abreu, L. A. S. (2011). Secagem e armazenamento de sementes de sorgo com alto e baixo teor de tanino. *Revista Brasileira de Sementes*, *33*(4), 699–710, https://doi.org/10.1590/s0101-31222011000400012.

Paixão, M. V. S., Grobério, R. B. C., Hoffay, A. C. N., Correa, A. C., and Cremonini, G. M. (2021). Ácido giberélico na germinação de sementes e desenvolvimento inicial de plântulas de mamoeiro. *Agrotropica*, *33*(2), 143–148, https://doi.org/10.21757/0103-3816.2021v33n2p143-148.

Pascoli, M., Lopes-Oliveira, P. J., Fraceto, L. F., Seabra, A. B., and Oliveira, H. C. (2018). State of the art of polymeric nanoparticles as carrier systems with agricultural applications: A mini review. *Energy, Ecology and Environment*, *3*(3), 137–148, http://dx.doi.org/10.1007/s40974-018-0090-2.

Pereira, A. E. S., Oliveira, H. C., and Fraceto, L. F. (2019). Polymeric nanoparticles as an alternative for application of gibberellic acid in sustainable agriculture: A field study. *Scientific Reports*, *9*(7135), 1–10, https://doi.org/10.1038/s41598-019-43494-y.

Pereira, A. E. S., Silva, P. M., Oliveira, J. L., Oliveira, H. C., and Fraceto, L. F. (2017). Chitosan nanoparticles as carrier systems for the plant growth hormone gibberellic acid. *Colloids and Surfaces B: Biointerfaces*, *150*, 141–152, https://doi.org/10.1016/j.colsurfb.2016.11.027.

Pereira, C., Cuquel, F. L., and Panobianco, M. (2010). Germination and storage of seeds of the bromeliad *Nidularium innocentii* (Lem.). *Revista Brasileira de Sementes*, *32*(2), 36–41, https://doi.org/10.1590/s0101-31222010000200004.

Peykani, L. S., and Sepehr, M. F. (2018). Effect of chitosan on antioxidant enzyme activity, proline, and malondialdehyde content in *Triticum aestivum* L. and *Zea maize* L. under salt stress condition. *Iranian Journal of Plant Physiology*, *9*(1), 2661–2670, https://doi.org/10.22034/ijpp.2018.545906.

Pinangé, D. S., Krapp, F., Zizka, G., Silvestro, D., Leme, E. M., Weising, K., and Benko-Iseppon, A. M. (2017). Molecular phylogenetics, historical biogeography and character evolution in *Dyckia* (Bromeliaceae, Pitcairnioideae). *Botanical Journal of the Linnean Society*, *183*(1), 39–56.

Pompelli, M. F. (2006). Germinação de *Dyckia encholirioides* var *encholirioides* (Bromeliaceae, Pitcairnioideae). *Floresta e Ambiente*, *13*, 1–9.

Purba, J. H., Srilaba, N., Wahyuni, P. S., and Wahyuda, P. B. (2021). Response of papaya (*Carica papaya* L.) seeds growth to the gibberellin (GA_3) and cow biourine. *Jurnal Agronomi Tanaman Tropika*, *3*(2), 148–158, https://doi.org/10.36378/juatika.v3i2.1386.

R Core Team. (2022). R: A language and environment for statistical computing. Foundation for Statistical Computing. Retrieved from: http://www.r-project.org.

Rajanaidu, N., and Ainul, M. M. (2013). Conservation of oil palm and coconut genetic resources. In M. N. Normah, H. F. Chin, and M. R. Barbara (Ed), *Conservation of Tropical Plant Species* (pp. 189–212). New York, USA: Springer.

Reflora (2023). Bromeliaceae in Flora do Brasil 2020. Jardim Botânico do Rio de Janeiro. Retrieved from: http://reflora.jbrj.gov.br/reflora/floradobrasil/FB66.

Rego, C. H. Q., Cardoso, F. B., Cotrim, M. F., Cândido, A. C. S., and Alves, C. Z. (2018). Ácido giberélico auxilia na superação da dormência fisiológica e expressão de vigor das sementes de graviola. *Revista de Agricultura Neotropical*, *5*(3), 83–86, https://doi.org/10.32404/rean.v5i3.2354.

Ribeiro, E. S., Oliveira, D. P., Souza, R. S., Pasa, M. C., and De Souza, A. T. M. (2012). Efeito da temperatura na germinação de sementes *Enterolobium contortisiliqunn* (Vell.) Morong-(Mimosoidae) e *Guazuma ulmifolia*-(Sterculiaceae). *Biodiversidade*, *11*(1), 23–30.

Shimizu, G. D., Marubayashi, R. Y. P., and Gonçaves, L. S. A. (2022). AgroR: Experimental Statistics and Graphics for Agricultural Sciences. R package version 1.2.1. Retrieved from: https://cran.r-project.org/web/packages/AgroR/index.html.

Silva, E. A. A., Oliveira, J. M., and Pereira, W. V. S. (2018). Sementes do Brasil - Produção e Tecnologia para Espécies. Cap 1. Fisiologia das sementes.

Souza, G. C. S., Araújo, L. K. G., Silva, R. C. S., Coelho, W. S. S., and Kiill, L. H. P. (2018). Avaliação de diferentes concentrações de ácido giberélico na germinação de sementes de *Rhaphiodon echinus* Shauer (Lamiaceae). *Informativo Abrates*, *28*(1), 1–5.

Tuan, P. A., Kumar, R., Rehal, P. K., Toora, P. K., and Ayele, B. T. (2018). Molecular mechanisms underlying abscisic acid/gibberellin balance in the control of seed dormancy and germination in cereals. *Frontiers in Plant Science*, *9*, 668, https://doi.org/10.3389/fpls.2018.00668.

Verdolin, L. G., Mariz, B. L., and Dias, L. L. C. D. (2021). Gibberellin and polyamines effects in growth and flowering of New Guinea impatiens. *Ornamental Horticulturae*, *27*(2), 247–254, https://doi.org/10.1590/2447-536x.v27i2.2255.

Xiong, M., Chu, L., Li, Q., Yu, J., Yang, Y., Zhou, P., Zhang, C., Fan, X., Zhao, D., Yan, C., and Liu, Q. (2021). Brassinosteroid and gibberellin coordinate rice seed germination and embryo growth by regulating glutelin mobilization. *The Crop Journal*, *9*(5), 1039–1048, https://doi.org/10.1016/j.cj.2020.11.006.

Yang, R., Xiao, C. F., Guo, Y. F., Ye, M., and Lin, J. (2018). Inclusion complexes of GA_3 and the plant growth regulation activities. *Materials Science and Engineering C*, *91*, 475–485, https://doi.org/10.1016/j.msec.2018.05.043.

Zhong, C., Patra, B., Tang, Y., Li, X., Yuan, L., and Wang, X. (2021). A transcriptional hub integrating gibberellin-brassinosteroid signals to promote seed germination in *Arabidopsis*. *Journal of Experimental Botany*, *72*(13), 4708–4720, https://doi.org/10.1093/jxb/erab192A.

Zhu, Z. J., Wang, H., Yan, B., Zheng, H., Jiang, Y., Miranda, O. R., Rotello, V. M., Xing, B., and Vachet, R. W. (2012). Effect of surface charge on the uptake and distribution of gold nanoparticles in four plant species. *Environmental Science & Technology*, *22*, 12391–12398, https://doi.org/10.1021/es301977w.

Zucchi, M. R., Santos, F. R., Rocha, E. C., Teixeira, I. R., and Pires, L. L. (2018). Water absorption and desiccation tolerance in *Bromelia reversacantha* Mez seeds. *Revista de Ciências Agrárias*, *41*, 151–160, https://doi.org/10.19084/RCA18143.

Received: August 14, 2023; accepted: October 23, 2023