PLANT GROWTH REGULATORS ON ATEMOYA SEEDS GERMINATION

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Recebido em: 2017.07.09 **Aprovado em:** 2018.08.07 **ISSUE DOI:** 10.3738/1982.2278.2832

SUMMARY: The aim of this study was to evaluate the performance of plant regulators on seed germination of different atemoya cultivars: Thompson, Gefner and PR-1. The seeds were extracted from ripe fruits and submitted to the following treatments: 50 mg L^{-1} gibberellic acid (GA_3), 10 ml kg^{-1} of seeds (Stimulate®), 6 ml kg^{-1} of seeds (Evolust®), and control. The experimental design was completely randomized in a factorial 4x3 (three seed treatments + control x three cultivars), using five samples of 25 seeds. The seeds were sowed on paper and placed in a germination chamber, using alternated temperature 20-30°C (16h - 8h) under light absence. There were evaluated the percentage of germinated seeds, normal seedlings, abnormal seedlings, and the germination speed index. The Thompson cultivar presented the highest percentage of germinated seeds, normal seedlings, and germination speed index, followed by the Gefner cultivar. Among the products, the GA_3 promoted greater germination percentage. Using GA_3 and Stimulate®, as a pre-germination treatment, provided high percentage of normal seedlings. The products utilized did not affect the germination speed index.

Keywords: *Annonaceae*. Propagation. Cultivars. Gibberellic acid. Biostimulant.

REGULADORES VEGETAIS SOBRE A GERMINAÇÃO DE SEMENTES DE ATEMÓIA

RESUMO: O objetivo deste trabalho foi verificar a atuação de reguladores e estimulantes vegetais na germinação de sementes de atemóia, cultivares Thompson, Gefner e PR-1. As sementes foram extraídas de frutos maduros e submetidas aos seguintes tratamentos: 50 mg L^{-1} de ácido giberélico (GA_3), 10 ml kg^{-1} de sementes do produto Stimulate®, 6 ml kg^{-1} de sementes do produto Evolust®, e controle. O delineamento experimental foi inteiramente ao acaso em esquema fatorial 4x3 (três tratamentos de sementes + controle x três cultivares), usando cinco repetições de 25 sementes. As sementes foram semeadas em papel germitest e colocadas em câmara de germinação, utilizando temperatura alternada de 20-30°C (16 – 8 horas) sob ausência de luz. As variáveis avaliadas foram: porcentagens de sementes germinadas, plântulas normais, plântulas anormais e índice de velocidade de germinação. Entre as cultivares avaliadas, a Thompson apresentou maiores percentuais de sementes germinadas, plântulas normais e índice de velocidade de germinação, seguida da Gefner que obteve valores intermediários. Dos produtos testados, o GA_3 proporcionou maior percentual de sementes germinadas. Os tratamentos de sementes com GA_3 e Stimulate® proporcionaram maiores percentuais de plântulas normais. Os produtos utilizados não influenciaram no índice de velocidade de germinação.

Palavras-Chave: *Annonaceae*. Propagação. Ácido giberélico. Bioestimulante.

INTRODUCTION

The *Annonaceae* family presents large numbers of genus and species; most of all are native from tropical and subtropical regions. Among the species that are destined to '*in natura*' consumption, the

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fruit growers are attracted by the atemoya (*Annona cherimola* Mill. X *Annona squamosa* L.) because its fruit is more palatable than other species, presenting sweet and aromatic taste, lower number of seeds, longer shelf life post-harvest, and higher productivity (MOSCA; LIMA, 2003).

The *Annona spp*. propagation is conducted through grafting and the rootstocks are produced through the seeds, being the atemoya between the recommended species (KAVATI, 1998). However, the germination of atemoya seeds, as the other *Annona spp* are slow and irregular due to the embryo immaturity (PAWSHE; PATIL; PATIL, 1997; SMET *et al.*, 1999), resulting in a long time to grow seedlings and rootstock.

Plant regulators play a key role on the seed germination process, mainly gibberellins, cytokinins, auxins, and ethylene acting as germination promoters, and abscisic acid as dormancy inducer. The giberelic acid (GA_3) has a striking effect on seed germination, stimulating the cell elongation and the synthesis of hydrolytic enzymes, which acts on starches, proteins and amino acids, releasing energy to plant growth (TAIZ; ZEIGER, 2006).

Cytokinins present substantial ability to promote cell division, acting on the processes of cell elongation and differentiation, especially when associated to the auxins. The cell elongation is also regulated by the auxins, because it actives the enzymes that work on the cell wall, causing the rupture of the cellulose microfibrils and increase the cell plasticity, which facility the water absorption and cellular growth (CASTRO; VIEIRA, 2001; TAIZ; ZEIGER, 2006).

Similarly, the use of plant stimulants on germination improves the seedling performance, accelerating the emergence and enhancing the seed potential. These chemical compounds, biologically active, can cease or decrease the impact of adverse factors on seed quality and performance (ARAGÃO *et al.*, 2003). According to Castro and Vieira (2001), the mixture of two or more plant regulators or substances, such as amino acids, nutrients and vitamins, is denominated biostimulant or plant stimulant and depending the concentration, composition, and proportion, these products can increase the plant growth.

There were performed some research about seed treatment applying GA_3 and biostimulants, aiming to break the dormancy and to reduce the period of seed germination for seedlings production of many species (STENZEL; MURATA; NEVES, 2003; FERREIRA *et al.*, 2007; SOUSA *et al.*, 2008; LIMA *et al.*, 2009). Stenzel, Murata e Neves (2003) verified that GA_3 in concentrations of 50 and 100 mgL^{-1}, promoted higher and faster germination in *Annona squamosa* and atemoya cultivars. The atemoya seeds from GA_3 imbibition at 50 mg L^{-1}, cultivars Gefner, PR-1 and PR-3, presented 67.5, 36.25 and 61.25% of germination, respectively. However, there was observed a decrease in germination percentage at 100 mg L^{-1} of GA_3. SOUSA *et al.* (2008) comparing pre-germination treatments during 12 hours using Stimulate® at 20 ml L^{-1}, GA_3 at 50 mg L^{-1} and 750 mg L^{-1}, observed the dormancy overcoming for *Annona squamosa* seeds in both GA_3 doses.

Regarding to the biostimulants, the Stimulate® treatment provided high percentage of seedling emergence and development for *Passiflora edulis* seeds using 16 ml kg^{-1} of seed (FERRERA *et al.*, 2007). The pre-immersion of *Artocarpus heterophyllus* seeds in GA_3 (200 mg L^{-1}) during two hours, Stimulate® at 5 ml L^{-1} and 10 ml L^{-1}, promoted 84% of germination at 10 ml L^{-1} of Stimulate® (LIMA *et al.*, 2009).

The GA_3 treatment associated to other plant regulators, such as biostimulants, are alternatives for overcoming dormancy and promoting seed germination of several species. However, the lack of information about the application and performance of biostimulants on *Annona spp*. seeds, justifies the necessity to develop research about atemoya cultivars. As a result, the aim of this study was to evaluate the performance of plant regulators and stimulators on seed germination of different atemoya cultivars: Thompson, Gefner and PR-1.

MATERIAL AND METHOD

The experiment was carried out at the Plant Production Department of the Northern Parana State University, Bandeirantes, Brazil. Ripped fruits of three atemoya cultivars: Thompson, Gefner and PR-1 were obtained from a commercial orchard in Assaí, Brazil. The seeds were extracted manually from the fruits; then, washed under running water on sieve, laid on absorbent sheet to dry out during three days, and packed in Kraft paper until sowing.

The determination of seed moisture content was performed utilizing the oven method at 105 ± 3 °C during 24 hours (BRAZIL, 2009), using four replicates of 100 seeds per cultivars and the results were expressed as percentage. The fresh and dry matters were obtained by weighing four subsamples of 100 seeds, and the result expressed by the subsamples arithmetic means.

Seeds from each cultivar were divided into four lots, immersed for six minutes in fungicidal solution of 0.2% metalaxyl-m + fluodioxonil (Maxin xl®) + 0.2% thiran 200 SC (Vitavax®) at 1:1. Then, the seeds were dried during 15 minutes and subjected to the following treatments: 50 mg L^{-1} of GA_3; 10 ml kg^{-1} seeds of Stimulate® (0.05 g L^{-1} of indolebutyric acid, 0.05 g L^{-1} of gibberellic acid, and 0.09 g L^{-1} of kinetin); 6 ml kg^{-1} seed of the commercial mixture Evolust® (0.1 g L^{-1} of indolebutyric acid, 0.05 g L^{-1} gibberellic acid, 0.2 g L^{-1} kinetin, 3 g L^{-1} of thiamine, and the nutrients B, S, Cu, Zn, Mn, Mo); and control.

The GA_3 treatment was conducted on paper (three sheets/plot), using the GA_3 solution equivalent to 2.5 of the paper-weight. The Stimulate® and Evolust® treatments were carried out through the mixture of seeds and products inside a Becker until total adherence, during six minutes.

Thereafter, there was performed the germination test on paper (Germitest®) according to Brazil (2009), placed in germination chambers (Biochemical Oxygen Demand - B.O.D.) at alternated temperatures of 20-30^{o}C (8 – 16 hours) and light absence, using five samples of 25 seeds per treatments. The evaluations were carried out at 7, 14, 21, 28 and 35 days after sowing, when the germination was stabilized. There was considered germinated seeds those with radicle length equal or greater than two millimeters. The characteristics evaluated were: percentages of germinated seeds, normal seedlings, abnormal seedlings, dormant seeds, dead seeds, and the germination speed index (GSI) by Maguire's formula (1962).

The experimental design was completely randomized arranged in a factorial 4x3, by combination of four seed treatments (GA_3, Stimulate®, Evolust®, and control) and three atemoya cultivars (Thompson, Gefner, and PR-1), using five replicates of 25 seeds. The data were submitted to the variance analysis by the Assistat program, and means compared by the Scott-Knott's test at 0.05 of probability. When necessary, the data were transformed to arcsen ((x/100) 1/2).

RESULT AND DISCUSSION

The data of moisture content, fresh and dry matters of 100 seeds are presented in the Table 1. For those characteristics, the PR-1 cultivar reached the highest values than Thompson and Gefner cultivars, which did not present differences in fresh and dry matter, only for the moisture content.

Table 1. Seed moisture content (%), fresh and dry matter of 100 seeds (g) in different cultivars of atemoya (*Annona cherimola* x *Annona squamosa*) seeds.

Cultivars	Seed moisture content	Fresh matter of 100 seeds	Dry matter of 100 seeds
Thompson	11,8 c	38,79 b	34,22 b
Gefner	12,3 b	36,24 b	31,79 b
PR-1	15,1 a	46,44 a	39,42 a
CV (%)	2,0	4,5	4,4

* Means followed by the same letters in the column do not differ statistically by Scott-Knott's test at 0.05.

In the table 2 are presented the data from the germination test. As can be seen, there was not observed interaction between the factors. However, there was noticed a significant difference between the cultivars, Thompson obtained the highest percentage of germinated seeds and the lowest percentage of dormant seeds when compared to PR-1, which presented lower germinate performance.

Table 2. Percentage of germinated seeds (GS), normal seedlings (NS) and abnormal seedlings (AN) of atemoya cultivars (*Annona cherimola* x *Annona squamosa*) submitted to different treatments

Treatments	7 days	14 days	21 days	28 days	35 days		
	GS	GS	GS	GS	GS	NS	AS
Thompson	9,4 A	51,4 A	55,0 A	56,6 A	56,8 A	42,8 A	12,2 A
Gefner	4,0 B	34,6 B	39,8 B	42,6 B	44,2 B	34,6 B	7,0 B
PR-1	1,2 C	20,6 C	24,0 C	27,6 C	27,6 C	21,0 C	5,2 B
GA_3	3,7 a	39,2 a	44,8 a	51,5 a	52,3 a	38,9 a	9,9 a
Stimulate®	3,7 a	36,3 a	41,6 a	42,9 b	42,9 b	36,0 a	5,9 a
Evolust®	5,6 a	34,1 a	36,0 a	37,1 b	37,3 b	26,1 b	10,9 a
Control	6,4 a	32,5 a	36,0 a	37,6 b	38,9 b	30,1 b	7,2 a
CV (%)	71,4	23,6	19,2	21,8	21,3	22,9	63,9

*Means followed by the same letters in the column do not differ statistically by Scott-Knott's test at 0.05.

For normal seedlings, was observed a similar effect between the cultivars. The development of germinated seeds to normal seedlings ranged from 75.4% in Thompson to 78.3% in Gefner. These results corroborate to Stenzel, Murata e Neves (2003), regarding the germination superiority of the cultivar Gefner over PR-1. During the germination test, the treatments studied indicate significant differences just from the 28 days after sowing, where the seeds submitted to the GA_3 treatment achieved superior germination performance. Stenzel, Murata e Neves (2003) obtained similar results for the Gefner and PR-1 cultivars, among other materials.

These authors worked with seed scarification before GA_3 immersion, during 24 hours, obtaining higher germination percentage than those noticed in the present study. This can be demonstrated through the results of germination percentage obtained at 35 days after sowing in both investigations, using the same GA_3 dosages (50 mg L^{-1}) for the Gefner (57.5 and 44.2%) and PR-1 (36.3 and 27.6%).

Due to the seeds remain intact, without scarification, and the GA_3 treatment have been performed on paper in order to respect the imbibition period of the seeds (FERREIRA *et al.*, 2006), must have contributed to the germination of this specie. However, the Stimulate® treatment did not differ from the GA_3 for the seedling characteristics, promoting larger number of normal seedlings, since cytokinin stimulates the shoot growth and the indolebutyric acid, the root growth, both present in its formulation.

It is possible to find information about Stimulate® treatment on germination and emergence of several species, but reports concerning *Annona spp.* are scarce (SOUSA *et al.*, 2008) and absent for the commercial mixture studied in this research. Thus, the results obtained in this study seems to be disadvantaged, because the seed treatments using stimulants consisted in the seed-product contact for only six minutes. However, Ferreira *et al.* (2007) working with *Passiflora edulis* seeds tested immersion in different Stimulate® concentrations (4, 8, 12, 16 and 20 ml kg^{-1} of seed), for just one minute. Contrasting to Lima *et al.* (2009), whose studied immersion of *Artocarpus heterophyllus* seeds in Stimulate® during two hours, at 5 and 10 ml kg^{-1}.

For the germination speed index (Table 3), the interaction between the factors was not significant. Regardless the treatments, the GSI data obtained by the cultivars presented similar effect as can be seen in germination percentage and normal seedlings, where the cultivar Thompson reached the highest rate followed by Gefner and PR-1. However, it is noticed that the Gefner obtained higher rate than PR-1, corroborating to Stenzel, Murata e Neves (2003). Regarding the treatment performance on the germination speed, there was not noticed significant difference between them. Similar results were obtained using these treatments on germination of *Artocarpus heterophyllus* seeds (LIMA *et al.*, 2009).

Table 3 - Germination speed index (GSI) of atemoya (*Annona cherimola* x *Annona squamosa*) seeds, cultivars Thompson, Gefner and PR-1, submitted to different treatments

Treatments	GSI
Thompson	1,143 A
Gefner	0,788 B
PR-1	0,461 C
GA_3	0,899 a
Stimulate®	0,789 a
Evolust®	0,741 a
Control	0,760 a
CV (%)	16,8

*Means followed by the same letters in the column do not differ statistically by Scott-Knott's test at 0.05.

CONCLUSION

The Thompson cultivar presents higher germination performance, when compared to Gefner and PR-1 cultivars. Among the products, the gibberellic acid (GA_3) provides a substantial percentage of germinated seeds. However, the products used in the present study do not affect on the germination speed index of atemoya seeds.

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