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COMBINED APPLICATION OF PLANT REGULATORS IN YOUNG FRUITS MODIFIES BIOMETRIC CHARACTERISTICS AND GERMINATION OF ATEMOEIRA SEEDS CV. 'THOMPSON'

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Abstract: The present work aims to analyze the effects of the application of plant regulators during fruit development on the biometric characteristics and germination of atemoya seeds (*Annona cherimola* Mill. x *Annona squamosa* L.) cv 'Thompson'. The fruits with an average of 2cm were treated with four concentrations of the biostimulant Stimulate®, equal to 500, 1000, 1500, 2000 and 0 (control) mL ha^{-1} i.a., at 14-day intervals for five months, under field conditions. After ripe fruit seeds were sown on germitest paper rolls and taken to the germination chamber where they remained in the dark, with a temperature alternating between 20°C for 8 hours and 30°C for 16 hours. For this purpose, a completely randomized experimental design was adopted, considering the treatments applied to the fruits in the field, distributed in five replications of 25 seeds each. The variables evaluated after the collection of the fruits were: number of seeds, fresh mass of seeds, dry mass of seeds, water content of seeds and in the germination test, it was considered: germination percentage, germination speed index and mean germination time. After the germination test, it was determined: number of dormant and dead seeds in tetrazolium. From the results, it can be seen that 1500 mL ha^{-1} in young fruits tends to increase the number of seeds, resulting in a lower water content. The seeds of the fruits treated with a concentration of 1000 mL ha^{-1}, took a shorter mean time to germinate. There is a trend between germination percentages and dormant seeds. It is concluded that, under the conditions of the experiment, the combined application of plant regulators in young fruits, discreetly modifies biometric characteristics and seed germination of atemoeira cv 'Thompson'.
Keywords: Annonaceae, Biostimulant, Biometric aspects, Germinability.

INTRODUCTION

The application of plant regulators allows plants to better express their productive genetic potential, since these are important metabolic activators. Currently, the combination of these products in fruits and vegetables has been widely used, due to the positive influence on the increase and quality of the harvest. They are compounds that accelerate plant growth, ranging from pollen tube development, attachment to the mother plant, to controlling maturation and stimulating seed germination (Mota Filho et al.,2012; Schaller et al., 2015; Fetter, 2018; European Parliament, 2019).

The plant regulator Stimulate® is a commercial product, consisting of indolylbutyric acid (auxin), kinetin (cytokinin) and gibberellic acid (gibberellins). This product is widely used in agriculture and modulates the responses of plants to the environment where they are found, such as absorption and use of nutrients, stimulating cell division, differentiation and elongation of the cell, organ or plant tissue (Castro, 2006; Long, 2019). However, the responses to the Stimulate® biostimulant depend on some factors such as: the species, part of the plant, development stage, concentration, interaction between other regulators and environmental factors (Campos et al., 2008).

Plant regulators have been widely used in order to overcome dormancy and increase the germination percentage of anonas species (Zucareli et al., 2009; Oliveira et al., 2010). However, the results with the use of Stimulate® applied directly to the seeds of anonas have not revealed a satisfactory effect in overcoming dormancy and improving germination, in addition to the use of plant regulators and their concentrations in different species, which are quite divergent.

Thus, research that helps in the knowledge of the use of plant regulators in atemoya production systems is quite relevant, as success in the orchard can justify field investment (Do Prado Verotti et al., 2019), in addition to the reflection of the effects of this product in a later stage, such as germination process. Among the factors that control the germination process are the endogenous levels of hormones, especially cytokinins and gibberellins, which promote changes in the physiological and biochemical state, culminating in the resumption of embryonic development (Fincher, 1989; Marcos Filho, 2005). Thus, the low percentage of seed germination of Annona species may be a consequence of the hormonal balance between germination promoters and inhibitors (Bewley et al., 2013; Shu et al., 2016). Therefore, the combined application of plant regulators, such as Stimulate®, can benefit the hormonal balance, given the vast amount of work that confirms such findings, including with anonas species (Corsato et al., 2012; Chagas et al., 2013).

Considering the importance of species of the Annonaceae family, which present dormancy establishment and need for sowing soon after dispersal, which makes their propagation difficult, both via seed and vegetatively, there is a need to know if the hormonal balance in young fruits and seeds in formation affects biometric and germination characteristics in atemoya plants. Based on the proposed, we hypothesized that the combined application of plant regulators in developing fruits modulates the hormonal balance and biometric characteristics, influencing the overcoming or improvement of the germination efficiency of anonas seeds. And aiming to evaluate the effects of the plant regulator 'Stimulate®' consisting of indolylbutyric acid (auxin), kinetin (cytokinin) and gibberellic acid (gibberellins), in young fruits and with different concentrations, affects biometric

characteristics and germination of atemoya seeds (*Annona cherimola* Mill. x *Annona squamosa* L.), cultivar 'Thompson'.

MATERIALS AND METHODS

The first stage took place with the application of Stimulate® on young fruits in field conditions conducted at "Sítio Paraizinho", located in the municipality of Pardinho/SP, at latitude of 23º 5' 3" South and altitude of 895 meters. (IBGE, 2015). The next step (germination test) was carried out in the Plant Physiology laboratories of the Botany Department, Biosciences Institute - UNESP, Botucatu Campus.

The treatments applied to young fruits consisted of a mixture containing auxin (4-indol-3-ylbutyric acid), cytokinin (kinetin) and gibberellin (gibberellic acid), obtained using the commercial product Stimulate®, Stoller do Brasil Ltda composed of 0.009% kinetin, 0.005% gibberellic acid and 0.005% indolylbutyric acid, in addition to 99.981% inert ingredients, at concentrations of 0, 500, 1000, 1500 and 2000 mL ha^{-1} i.a. In all treatments, 0.25% adhesive distributor (Natur'1 Óleo®) was added to increase the adhesion of the regulator to the aerial part of the atemoya plants, and acidification of the solution was carried out, maintaining the final pH of the mixture between 4.0 and 5.0 using P-51® (1% nitrogen and 51% phosphoric acid), according to Do Prado Verotti et al. (2019).

In field conditions, a randomized block experimental design was adopted, with five treatments and five replications, totaling 25 atemoya trees (*Annona cherimola* x *Annona squamosa*), cultivar 'Thompson'. In each tree, 10 fruits were marked, with an average length between 4.00cm and 6.00cm and diameter, between 3.00cm and 5.00cm, at the beginning of the treatments application. Eight applications were carried out on the marked fruits, at 14-day intervals, that is, until ripening.

The fruits, after ripe (peel of green color and seeds with tegument presenting dark brown color), were collected, pulped manually with the aid of a sieve and running water. Then, it was determined from 10 repetitions of standardized fruits according to their dimensions and weight, the number, water content, fresh and dry mass of the seeds. The seeds were dried in an oven at 105°C±3°C to determine the water content, which, in turn, was obtained by the difference between fresh and dry mass of the seeds.

The germination test partly followed the recommendations of Lorenzi (2016). The ripe fruits were kept at rest after collection, which facilitates the processing of the seeds, carried out in the laboratory with the help of a sieve and running water. The seeds were dried in a laboratory environment (25°C±2°C) until reaching between 10% and 12% of moisture, in order to improve their response to treatments to overcome dormancy, before the installation of the experiments. Afterwards, the seeds were disinfected with 2% sodium hypochlorite for 3 minutes and washed in running water to avoid the development of pathogens. Then, sowing was carried out in a germination paper roll, germitest type, using double leaves. The amount of distilled water used for moistening was 2.5 times the mass of the paper, as recommended by Brasil (1992). The material was packed in transparent plastic bags and taken to the germination chamber where they remained in the dark, with a temperature alternating between 20ºC for 8 hours and 30ºC for 16 hours, according to Ferreira et al. (2002c). During the germination period, fungal treatments were used with benomyl solution at a concentration of 2 g L^{-1} i.a applied every 15 days.

A completely randomized experimental design was adopted, considering the

concentrations of Stimulate® applied to the fruits in the field, distributed in five replications of 25 seeds each, totaling 125 seeds per treatment. Daily observations were carried out for 124 days and the seeds that presented radicle protrusion were considered germinated.

The variables evaluated were: germination percentage [%G], considering germinated the seeds that emitted at least 2mm of primary root (HADAS, 1976), germination speed index [GSI] (Silva; Nakagawa, 1995), mean germination time (MGT) (Edmond; Drapala, 1958), using the following equations:

$GSI = (C_1/T_1\text{-}A+C_2/T_2\text{-}A...+Ci/Ti\text{-}A)x100/Nx100/P.$

Where:

C1, C2 and Ci: daily germination count;

T1, T2 and Ti: time;

P: germination percentage;

A: period during which germination takes place;

N: number of seeds under test.

$$MGT = \frac{G1T1+G2T2+...+GnTn}{G1+G2+...+Gn}$$

Where:

G1, G2 and Gn: number of germinated seeds;

T1, T2 and Tn: germination time, respectively, of G1, G2 and Gn.

At the end of the germination test, it was observed number of dormant and dead seeds in tetrazolium.

The data were submitted to analysis of variance and the means were compared by the Tukey test at a 5% significance level (Zar, 2010).

RESULT AND DISCUSSION

Table 1 shows the average number, fresh and dry masses and water content of seeds obtained from fruits after the application of different combinations and concentrations of 'Stimulate®' plant regulators. The application of Stimulate® concentrations did not change these variables. Despite this, from the results, we can see that 1500 mL ha^{-1} tends to increase the number of seeds, which may reflect in the lower water content. Seed formation represents a critical stage in the life cycle of plants and is controlled genetically and by hormonal action, such as auxins (indolylbutyric acid (IAA)), gibberellins (GA), cytokinins and abscisic acid (ABA), involving an ordered sequence of morphological, physiological and biochemical changes that occur from the fertilization of the ovum to the detachment of the mature seed from the mother plant (Beltrani; Paoli, 2003; Waterworth; Bray, 2007).

The fresh seeds contained on average about 30% of water and after drying it reduced to 15%, regardless of the application with regulator or not (Table 1). Seeds that have a high water content can reflect on the formation of quality seedlings, since water is required for cell division, as well as the interaction between cytokinin (transzeatin), AIA and GA (Lulsdorf et al., 2013), ensuring control of the process of cell division and elongation, in the initial stages of seed development (Zhao, 2010), especially when the embryo's tissues are not yet fully differentiated (Bajguz; Piotrowska, 2009). Corsato (2014) found that even low water contents (10 and 5%), the seeds of *Anona emarginata* (Schltdl.) H. Rainer (Fria-terra araticum) lost germination capacity and significantly reduced energy reserves, mainly after 120 and 180 days. In addition, these seeds when they presented initial water content (59%) and reduced to 20 and 15% did not germinate.

Concentration	Number of seeds	Fresh seed pasta	Dry seed mass	Seed water contente
(mL ha^{-1})	(Items.)	(g)	(g)	(%)
0	13.80a±0.920	11.28a±0.205	7.06a±0.120	4.21a±0.207
500	16.23a±0.510	12.37a±0.431	7.66a±0.170	4.71a±0.381
1000	15.53a±1.710	12.09a±0.127	7.73a±0.088	4.35a±0.153
1500	18.60a±1.600	11.85a±0.579	7.91a±0.386	3.94a±0.269
2000	14.62a±0.750	11.48a±0.229	7.53a±0.287	3.95a±0.155
CV	19.25	7.53	14.64	14.64
AVERAGE	15.75	11.81	4.23	4.23
DMS	5.87	1.68	1.17	1.173

Means followed by the same letter in the column do not differ by Tukey's test at 5% probability.

Table 1 - Number, mass and water content of atemoya seeds (*Annona cherimola* Mill. x *A. squamosa* L.), cultivar 'Thompson', from fruits treated with different concentrations of plant regulators. Medium values.

Concentration	Germination	Germination speed index	Mean germination time	Number of dormant seeds	Number of dead seeds
(mL ha^{-1})	(%)	-	(Days)	(Items.)	(Items.)
0	34.40a±2.677	0.26a±0.037	46.54ab±1.716	8.60a±0.820	4.20a±0.650
500	33.60a±1.824	0.22a±0.028	57.49ab±3.485	7.40a±0.660	4.00a±0.490
1000	33.60a±2.427	0.27a±0.023	45.08b±3.594	8.40a±0.450	5.40a±0.530
1500	31.20a±0.716	0.18a±0.008	52.04ab±2.580	7.80a±0.910	4.00a±0.400
2000	35.20a±3.078	0.17a±0.018	58.80a±1.515	8.00a±0.560	5.20a±0.52
CV	17.09	27.10	13.07	20.87	30.35
AVERAGE	33.60	0.22	51.98	8.04	4.56
DMS	10.86	0.11	12.86	3.25	2.68

Means followed by the same letter in the column do not differ by Tukey's test at 5% probability.

Table 2 - Germination (%), germination speed index, mean germination time, number of dormant and dead seeds in tetrazolium of atemoya (*Annona cherimola* Mill. x *A. squamosa* L.), cultivar 'Thompson', from fruits treated with different concentrations of plant regulators. Medium values.

However, the water content of the seeds did not favor the other variables analyzed, such as the germination percentage (%) and germination speed index (GSI), possibly due to the lower development of these seeds, reflecting in large amounts of seeds per fruit, which reached 34.8%, negatively affecting carbohydrate reserves, which provide greater vigor to the seeds and contribute as a source of energy in germination (Table 2). In addition, different chemical compositions and/or mobilization of seed reserves can determine the potential for vigorous seedling formation (Santos et al., 2019). Braga et al. (2010) working with seeds of *Annona cherimola x A. squamosa,* obtained the highest germination percentages by applying 520 mg L^{-1} of GA_3 or 329 mg L^{-1} of GA4+7+N-(phenylmethyl)-aminopurine, respectively, 89.44 and 95.45%, while the use of 'Stimulate'' biostimulant consisting of Gibberellin+Citokinin+Auxin, did not promote an increase in the germination process.

In the mean germination time (MGT), it was observed that seeds of fruits treated with a concentration of 1000 mL ha$^{-1,}$ presented

a shorter time to germinate, differing statistically only from the concentration of 2000 mL ha^{-1} that had a longer germination time (Table 2).

At 124 days after the germination test, no difference between the number of dormant seeds and the number of dead seeds in tetrazolium (Table 2).

Trends for germination percentages and dormant seeds can be seen (Table 2). Generally, in seeds that have mechanical restriction to germination, only an increase in GA levels may be sufficient to overcome dormancy. This hormone can be synthesized in the radicle from where it is released to act on the degradation of embryo envelopes (Kucera et al., 2005).

In the literature there are reports that seeds of *Annona cacans* Warm. (Annonaceae) sowing without treatments, dormancy overcoming reaches a maximum of 21% of germination in a period of 23 (twenty-three) weeks (Suganuma et al., 2008). This demonstrates the importance of clarifying the dormancy mechanisms of the species, with which reproduction strategies can be defined.

In general, it must be added that it was not possible to indicate an optimal concentration for the regulators, within the concentrations studied in the present experiment, because some characteristics showed a linear effect. However, discrete benefits for the variables studied occurred when the regulators were used at concentrations of 1000 mL ha^{-1} of Stimulate®. Thus, the species demonstrates another dormancy mechanism not yet reported in this family (Dalanhol et al., 2013). In addition, it may be that the application of scarification associated with treatments could result in greater germinability of atemoeira seeds.

CONCLUSION

It is concluded that, under the conditions of the experiment, the combined application of plant regulators in young fruits, discreetly modifies biometric characteristics and seed germination of atemoeira cv 'Thompson'. Its use can be enhanced according to the way of application, concentration tested and association with other procedures, such as scarification.

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