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GERMINATION OF ATEMOYA (*Annona Cherimola* Mill. x *A. squamosa* L.) CV. GEFNER SEEDS SUBJECTED TO TREATMENTS WITH PLANT GROWTH REGULATORS

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ABSTRACT
The aim of this study was to evaluate the germination of atemoya (*Annona cherimola* Mill. x *A. squamosa* L.) cv. Gefner seeds treated with plant growth regulators. Experimental design was completely randomized, with four replicates of 25 seeds per plot and 40 treatments. Seeds were subjected to GA_3 (0, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, and 3000 mg L^{-1} a.i.), GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine (0, 12.5, 25, 50, 75, 100, 200, 300, 400, 500, 1000, 1500, 2000, 2500, and 3000 mg L^{-1} a.i.) and CK+GA+AX (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mL Kg^{-1} seed). The germination percentage and speed index (GSI), besides the percentage of dormant and dead seeds and normal and abnormal seedlings were subjected to variance and regression analyses. The estimated concentration of 520 mg L^{-1} GA_3 led to 89.44% germination and 5.21 GSI (548.80 mg L^{-1} estimated concentration). GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine led to 95.45% germination (329.14mg L^{-1}) and 3.97 GSI (332.99 mg L^{-1}). CK+GA+AX led to 50.78% germination (2.77 mL Kg^{-1}) and 2.88 GSI (2.97 mL Kg^{-1}). GA_3 and GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine were more effective than CK+GA+AX in the germinative process of atemoya cv. Gefner seeds.

KEYWORDS: Annonaceae, gibberellin, cytokinin, auxin, seed dormancy.

INTRODUCTION

The family Annonaceae includes around 135 genera and 2500 species, some of which are commercially important, such as sweetsop (*Annona squamosa* L.), atemoya (*A. cherimola* Mill. x *A. squamosa* L.) and soursop (*A. muricata* L.) (Donadio, 1997, Chartro et al., 2004). Annonaceae plants are mostly propagated through grafting. Rootstocks for atemoya have been originated from seeds of several species, even from atemoya itself due to problems of incompatibility with certain species such as pond apple (*A. glabra* L.), mountain soursop (*A. montana* Macf.), soursop (*A. muricata*), and biriba (*Rollinia mucosa*) (Kavati, 1992, Stenzel et al., 2003).

Seedling production begins, therefore, from rootstock seed germination, which is slow and uneven due to the immature embryo of slow development, besides the abscisic acid concentration and the tegument impermeability and resistance (Pawshe et al., 1997, Smet et al., 1999), resulting in up to 24 months added to the rootstock formation time (Bezerra and Lederman, 1997). Thus, the use of plant growth regulators may improve the germination of these seeds. To break dormancy and/or increase the germinative process, the hormonal balance between germination inhibitors, such as abscisic acid (ABA), and promoters, such as GA_3, must be changed (Weaver, 1997, Kigel and Galili, 1995). Gibberellins (GAs) activate the embryonic vegetative growth, weakens the endosperm layer that involves the embryo and restricts its growth, and mobilizes the energetic reserves from the endosperm of cereals (Bewley, 1997, Bradford et al., 2000, DeCastro and Hilhorst, 2004, Taiz and Zeiger, 2006). Cereal embryo synthesizes and releases GA during the germination, which leads to the production and/or secretion of several hydrolytic enzymes involved in the solubilization of reserves, including α and β-amylase (Taiz and Zeiger, 2006). Cytokinins induce cell division, participating in cell elongation and differentiation, especially when they interact with auxins (Arteca, 1995). Copeland and McDonald (1995) stated that the action of kinetin on germination is related to the membrane permeability. Auxins act on the plasticity of the cell wall, providing the latter with irreversible elongation (Arteca, 1995, Taiz and Zeiger, 2006).

Plant growth regulators may act alone or combined with other regulators (Davies, 2004). In this case, the mixture of two or more plant growth regulators or between plant growth regulators and other substances is named biostimulant (Vieira and Castro, 2001).

Pawshe et al. (1997) applied 100 mg L^{-1} GA_3 on *A. esquamosa* L. seeds and obtained 56% germination. Valenzuela and Osório (1998) reported the highest mean germination (55.4%) of *A. reticulata* seeds by using 10000 mg L^{-1} GA_3. Smet et al. (1999) detected the highest germination value (74.5%) when 1000 mg L^{-1} GA_3 were applied on *A. cherimola* Mill seeds. In studies with *Annona squamosa* L., Ferreira et al. (2002) obtained 77% germination under 250 mg L^{-1} GA_3, whereas Stenzel et al. (2003) detected 75.0% germination under 50 mg L^{-1} GA_3. As regards atemoya seeds, Stenzel et al. (2003) reported 67.5% germination for the cultivar Gefner, 36.25% for PR–1 and 61.25% for PR-3 after the application of 50 mg L^{-1}, in addition, Oliveira (2004) obtained 80% germination

by using 500 mg L^{-1} GA_3 on seeds of atemoya cv. Gefner. Silva et al. (2007) detected 43% germination when 500µM GA_{4+7} were applied on *A. crassiflora* seeds.

This study aimed to evaluate the germination of atemoya (*A. cherimola* Mill. x *A. squamosa* L.) cv. Gefner seeds treated with GA_3, GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine and CK+GA+AX.

MATERIAL AND METHODS

The experiment was carried out in the Seed Lab of the Department of Plant Production, School of Agronomical Sciences (FCA), São Paulo State University (UNESP), Botucatu Campus, São Paulo State (SP), Brazil. Atemoya (*A. cherimola* Mill. *x A. squamosa* L.) cv. Gefner fruits were obtained in a commercial production area from Angatuba Municipality, SP. The seeds were extracted, washed in tap water, immersed in hypochlorite (10%) for 1h, washed in autoclaved distilled water, immersed in oxytetracycline (100 mg L^{-1}) for 20 min, and washed again. After the phytosanitary treatment, the seeds were kept on a laboratory bench for seven days and then stored in a refrigerator for 15 days until the beginning of the experiments.

Experimental design was completely randomized, with 40 treatments and four replicates of 25 seeds per plot. Treatments consisted of the following plant growth regulators: GA_3 (0, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, and 3000 mg L^{-1} a.i.), GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine (0, 12.5, 25, 50, 75, 100, 200, 300, 400, 500, 1000, 1500, 2000, 2500, and 3000 mg L^{-1} a.i.), and CK+GA+AX (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mL kg^{-1} seed).

The commercial product Pro-Gibb® was used as source of GA_3 and was composed of 10% gibberellic acid (GA_3) and 90% inert ingredients in the form of soluble powder. GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine was provided by the biostimulant Promalin® and was composed of a mixture of GA_4 + GA_7 (1.8%) + N-(phenylmethyl)-1H-purine-6-amine (1.8%) and 96.4% inert ingredients. The biostimulant Stimulate® was used as source of CK+GA+AX and was composed of kinetin (90 mg L^{-1}, 0.009%), gibberellic acid (50 mg L^{-1}, 0.005%), indolebutyric acid (50 mg L^{-1}, 0.005%) and inert ingredients (99.981%).

Seeds were treated with the plant growth regulators GA_3 and GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine by immersion for 36h (Ferreira et al., 2006), under constant aeration. The mixture CK+GA+AX was directly applied on the seeds in plastic bags, which were vigorously shaken for 2 min in order to homogenize the adsorption of the product into the tegument. Then, the seeds received fungal treatment with (3a*R*,7a*S*)-2-[(trichloromethyl)sulfanyl]-3a,4,7,7a-tetrahydro-1*H*-isoindole-1,3(2*H*)-dione (Captan 50 P.M.) at 0.5% and were allowed to germinate in germitest paper roll moistened with 2.5-fold more distilled water than the paper weight (Brasil, 1992). Afterwards, they were kept in a BOD chamber at alternate temperature (20ºC/16h and 30ºC/8h) in the dark (Oliveira, 2004).

The following variables were evaluated: percentage of germination (%G), dead seeds (%DeS), dormant seeds (%DoS), normal seedlings (%NS) and abnormal seedlings (%AS), besides germination speed index (GSI). Seeds were considered germinated when they presented a primary root of 2mm length (Rehman et al., 1996). The tetrazolium test was used to detect the percentage of dead and dormant seeds (Oliveira et al., 2005). The percentage of normal and abnormal seedlings was obtained according to Brasil (1992). GSI was quantified by using the formula of Maguire (1962).

The results were subjected to variance (F-test) and regression analyses. The transformation $\arcsin\sqrt{x/100}$ was adopted for percentage values (Banzatto and Kronka, 1992).

RESULTS AND DISCUSSION

As regards germination percentage (%G) and germination speed index (GSI), there were significant effects according to the analysis of variance for the concentrations of GA_3 and GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine applied on atemoya seeds, which was not observed for the mixture CK+GA+AX (Table 1).

The quadratic regression model was significant for germination percentage and GSI (Figure 1). The models were fit from 0 to 1000 mg L^{-1} GA_3 and from 0 to 500 mg L^{-1} GA_{4+7}+ N-(phenylmethyl)-1H-purine-6-amine. Higher concentrations could not be fit, since the curves stabilized. According to the regression analysis, 520 mg L^{-1} GA_3 led to the highest germination (89.44%), whereas the control resulted in 52% germination (Figure 1A). From the control to the maximum concentration (520 mg L^{-1} GA_3), the curve had a crescent tendency, followed by a deep reduction up to 1000 mg L^{-1}, consequently, germination was around that observed for the control (52%). When GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine (Figure 1B) was used, the curve also had a crescent tendency, and germination peaked (95.45%) under the estimated concentration of 329.14 mg L^{-1}, followed by a decrease. The values obtained with CK+GA+AX were lower than those observed with the previously mentioned plant growth regulators, demonstrating less efficiency in inducing germination. The maximum point was obtained with 2.77 mL Kg^{-1} seeds, which resulted in 50.78% germination (Figure 1C).

As regards GSI, the highest values were detected under the estimated concentrations of 548.80 mg L^{-1} GA_3 (Figure 1D), 332.99 mg L^{-1} GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine (Figure 1E) and 2.97 mL CK+GA+AX Kg^{-1} seeds (Figure 1F), which resulted in 5.21, 3.97 and 2.88 GSI, respectively. The estimated concentrations of GA_3, GA_{4+7}+ N-(phenylmethyl)-1H-purine-6-amine and CK+GA+AX increased the germination percentage by 41.9%, 43.4% and 1.5%, respectively, relative to control. Considering GSI, GA_3 led to a 42.5% increase, and GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine to a 1.5% increase, however, CK+GA+AX reduced GSI by 5.6%, relative to control.

Table 1: Analysis of variance regarding percentage of germination (%G), normal seedlings (%NS), abnormal seedlings (%AS), dead seeds (%DeS), and dormant seeds (%DoS), besides germination speed index (GSI) for atemoya cv. Gefner

seeds subjected to treatments with concentrations of GA_3, GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine and CK + GA + AX.

		% G		GSI		% NS		% AS		% DeS		% DoS	
GA_3 (mg L^{-1})													
	F	4.03	**	4.00	**	1.62	NS	0.80	NS	2.70	**	8.36	**
	CV	14.34		15.72		19.60		26.19		43.02		18.58	
	MSD	25.71		1.59		19.12		22.37		20.55		10.68	
GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine (mg L^{-1})													
	F	6.12	**	3.53	**	2.93	**	0.78	NS	1.53	NS	3.52	**
	CV	13.39		14.25		25.75		23.94		60.55		26.67	
	MSD	24.18		3.32		25.60		20.49		24.44		16.61	
C K+GA+AX (ml kg^{-1})													
	F	1.23	NS	1.22	NS	2.83	*	0.16	NS	2.18	*	0.70	NS
	CV	13.97		13.50		22.18		21.60		22.81		51.05	
	MSD	14.91		3.11		12.57		17.87		24.61		18.71	

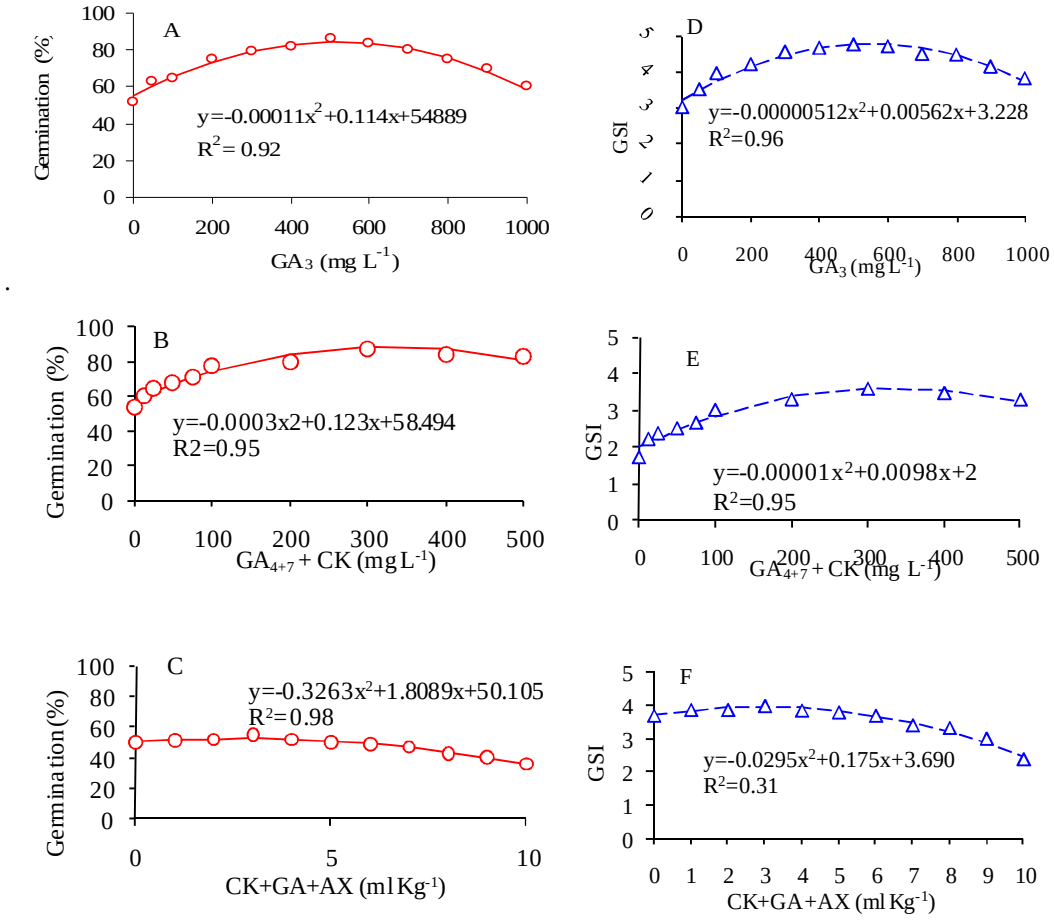
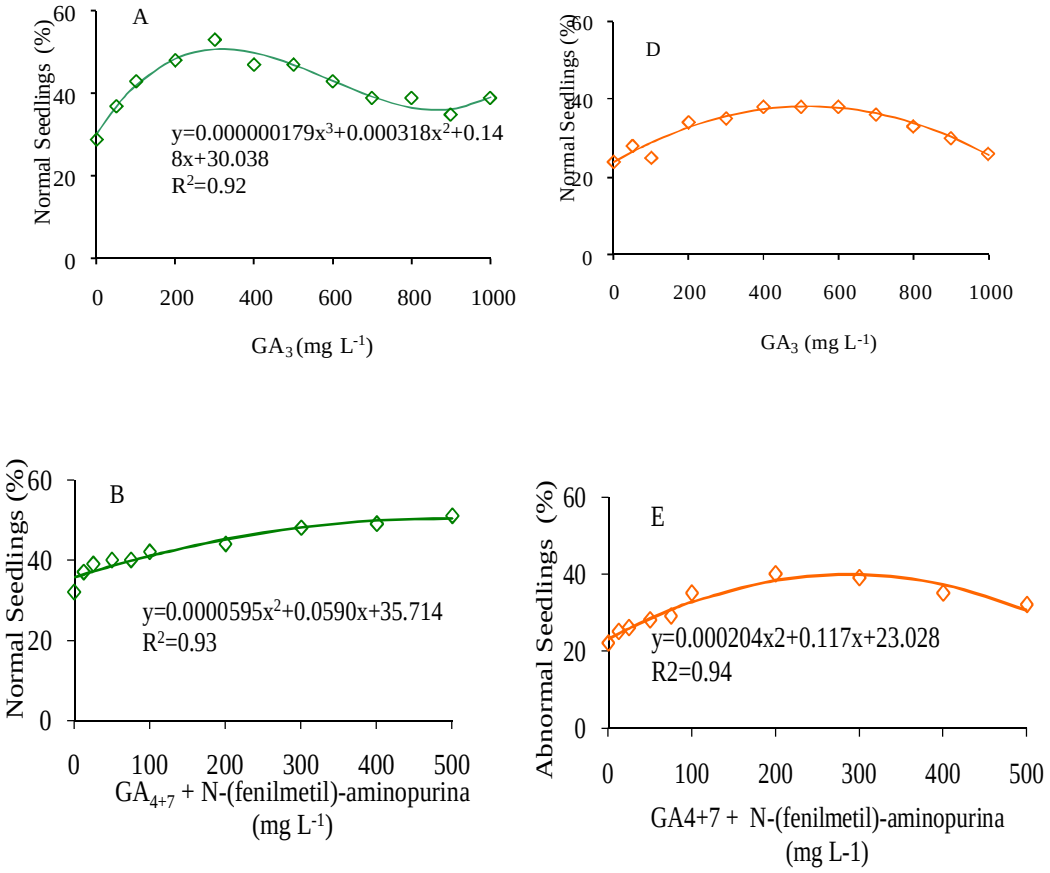


Figure 1. Quadratic regression models regarding germination percentage (%G) (A, B, C) and germination speed index (GSI) (D, E, F) for atemoya (*Annona cherimola* Mill. x *A. squamosa* L.) cv. Gefner seeds treated with plant growth regulators.

GA_3 action on atemoya seed germination was also observed by Stenzel et al. (2003), who obtained 67.5%, 36.25% and 61.25% germination for the cultivars Gefner, PR-1 and PR-3, respectively, by using 50 mg L^{-1} GA_3. However, those authors observed lower germination percentage under 100 mg L^{-1}, which was detected in the present study only from 520 mg L^{-1} GA_3. Similarly, Oliveira (2004) observed higher germination percentage for atemoya cv. Gefner seeds by using 500 mg L^{-1} GA_3, which resulted in 80% germination. Gibberellin stimulation is in agreement with Hopkins (1999) and Taiz and Zeiger (2006), since it may have interfered with the

synthesis of enzymes such as α and β–amylase and with the mobilization of reserves stored in the endosperm to the growth regions, which led to cell elongation of embryonic tissues and resulted in higher germination percentage and speed. Also, Silva et al. (2007) suggested increased germination due to the effect of GA_{4+7} on the induction of endo-b-mannanase in *Annona crassiflora* Mart. In the present study, increased germination (Figure 1 B, E) was also detected when plant growth regulators from two hormonal groups were combined, gibberellins (GA_{4+7}) and cytokinins [N-(phenylmethyl)-1H-purine-6-amine], which agrees with Fraga (1982), who stated that cytokinins are complementary to gibberellins in the induction of enzymatic processes when they are blocked by inhibitors such as abscisic acid and coumarin. Thus, lower concentrations can be used, since similar germination percentages (95.45% and 89.44%) were obtained under lower concentrations of GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine (329.14 mg L^{-1}), compared to those of GA_3 (520 mg L^{-1}). The synergistic effect between cytokinin and gibberellin was also reported by Leonel and Rodrigues (1995) for 'Cravo' lemon seed germination (92.12%) and by Ono et al. (1993) for 'Volkameriano' lemon (*Citrus volkameriana*) (89%), both with GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine.

As regards CK+GA+AX (Figure 1C, F), there was an increase in germination percentage and speed, relative to control. However, such results were lower than those obtained when GA_3 and GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine were used. When compared to the control, these results agree with those of Neto et al. (2007), who observed a significant increase in the germination of genipap (*Genipa americana* L.) seeds treated with Stimulate®, and those of Castro and Vieira (2001) for soybean (*Glycine max* (L.) Merrill), bean (*Phaseolus vulgaris* L.) and rice (*Oryza sativa* L.) seeds. The analysis of variance indicated a significant effect on normal seedling percentage under application of GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine and CK+GA+AX (Table 1). The quadratic regression model was significant for normal seedlings treated with biostimulants and cubic for those subjected to GA_3 (Figure 2).



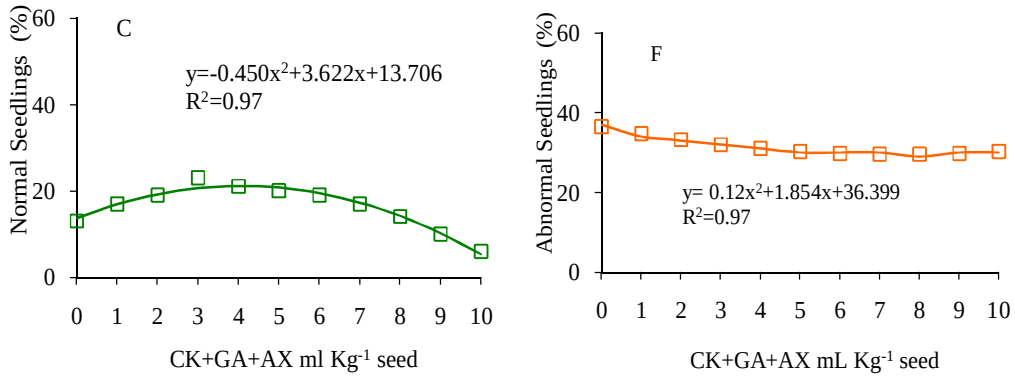


Figure 2. Quadratic and cubic regression models regarding percentage of normal (%NS) and abnormal (%AS) seedlings for atemoya (*Annona cherimola* Mill. x *A. squamosa* L.) cv. Gefner seeds treated with plant growth regulators.

The concentrations of 495.80 mg L^{-1} GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine (Figure 2B) and 4.07 ml Kg^{-1} CK+GA+AX (Figure 2C) led to the highest quantity of normal seedlings, equal to 52.8% and 31.2%, respectively, based on the regression analysis. These concentrations increased normal seedling percentage by 20% and 14.2%, relative to control, the values of which were 32% and 13%. Under GA_3 application, the cubic regression model presented a coefficient of determination of 92%, with two critical points, a maximum (391.19 mg L^{-1}) and a minimum point (866.17 mg L^{-1}). The former led to 73.7% normal seedlings, which represented a 44.7% increase, relative to control (29%). A much lower increase (10%, 39% normal seedlings) was detected for the minimum point. It must be emphasized that there was an increase in this variable, relative to control, even for this minimum point (Figure 2A). This statement disagrees with that of Ferreira et al. (2002), who did not detect differences in the percentage of *Annona squamosa* L. normal seedlings from seeds subjected to the application of 250 mg L^{-1} GA_3 on seeds. Conversely, Oliveira (2004) observed a linear increase in the percentage of normal atemoya seedlings from 0 to 1000 mg L^{-1} GA_3.

As regards abnormal seedlings, the quadratic regression model presented coefficient of determinations equal to 0.93 for GA_3, 0.94 for GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine, and 0.97 for CK+GA+AX (Figure 2 D,E,F). The estimated concentrations of 517.76 mg L^{-1} GA_3, 495.80mg L^{-1} GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine, and 7.48 mL Kg^{-1} CK+GA+AX led to the highest quantity of abnormal seedlings, equal to 39.0%, 32.0% and 30.0%, respectively, based on the regression analysis. Under application of GA_3 and GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine, the effect of these concentrations increased by 16% and 10% the abnormal seedling percentage, relative to control, the values of which were 23% and 22%, respectively, whereas CK+GA+AX decreased it by 0.7%, relative to control (37%). These results disagreed with those obtained by Ferreira et al. (2002), who did not detect significant effects on abnormal seedling percentage for sweetsop (*Annona squamosa* L.) subjected to 250 mg L^{-1} GA_3, and Oliveira (2004), who did not observe an increase in the percentage of atemoya cv. Gefner abnormal seedlings under application of GA_3 and ethephon.

The values regarding dormant and dead seeds could not be fit by regression models, although a significant effect was observed for dormant seed percentage under the application of GA_3 and CK+GA+AX and for dead seeds subjected to GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine, according to the F-test.

CONCLUSION

GA_3 and GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine were more effective than CK+GA+AX on the germinative process of atemoya (*Annona cherimola* Mill. x *A. squamosa* L.) cv. Gefner seeds.

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