ISSN 0100-2945 http://dx.doi.org/10.1590/0100-29452016234

OVERCOMING SEED DORMANCY IN *Annona macroprophyllata* AND *Annona purpurea* USING PLANT GROWTH REGULATORS[1]

GISELA FERREIRA[2], IVAN DE-LA-CRUZ-CHACÓN[3], ALMA ROSA GONZÁLEZ-ESQUINCA[4]

RESUMO-Sementes de Annonaceae são conhecidas por possuírem mecanismos de dormência que variam de impermeabilidade de tegumento até à dormência fisiológica. Desta forma, o objetivo deste estudo foi avaliar os efeitos da giberelina GA_3 e de GA_{4+7}+ a citocinina benziladenina (GA+BA) na superação da dormência de sementes de *Annona macroprophyllata* Donn. Sm (papausa) e *Annona purpurea Moc. &* Sessé ex Dunal (chincuya). O experimento foi realizado com a aplicação de GA_3 e GA_{4+7} + BA nas concentrações de 0; 200, 400, 500, 600, 800 e 1.000 mg L^{-1}. O uso dos reguladores resultou na quebra da dormência em ambas as espécies. Contudo, a aplicação da mistura GA_{4+7} + BA resultou em maiores incrementos na germinação de *A. macroprophyllata* do que em *A. purpurea*. Os tratamentos que promoveram as maiores porcentagens de germinação foram 200 mg L^{-1} de GA_{4+7} + BA para *A. macroprophyllata* (77%) e 200 mg L^{-1} de GA_{4+7} + BA e 500 mg L^{-1} de GA_3 para *A. purpurea* (30% e 29%, respectivamente). O índice de velocidade, o tempo médio e a frequência de germinação foram diferentes para ambas as espécies e com ambos os reguladores. Conclui-se que, apesar de GA_3 e GA_{4+7} + BA promoverem a germinação, a mistura GA_{4+7} + BA foi mais efetiva que GA_3 para quebrar a dormencia de ambas as espécies. Além disso, sementes de *A. purpurea* apresentam dormência mais difícil de ser superada que *A. macroprophyllata.*

Termos de indexação: Annonaceae, GA_3, GA_{4+7}, N-(fenilmetil)-aminopurina, germinação-Annonaceae, *Annona diversifolia*.

SUPERAÇÃO DA DORMÊNCIA DE SEMENTES DE *Annona macroprophyllata* E *Annona purpurea* COM O USO DE REGULADORES VEGETAIS

ABSTRACT-Some Annonaceae seeds are known to exhibit dormancy mechanisms ranging from possible seed coat impermeability to physiological dormancy. Thus, the aim of this study was to evaluate the effects of gibberellin (GA) GA_3 and GA_{4+7} + benzyladenine (GA_{4+7} + BA) application in seeds of *Annona macroprophyllata* Donn. Sm (papausa) and *Annona purpurea Moc. &* Sessé ex Dunal (chincuya). The experiment was performed by the application of GA_3 and GA_{4+7} + BA on seeds in concentrations of 0, 200, 400, 500, 600, 800 and 1000 mg L^{-1}. The regulators broke the dormancy of both species. However, application of the GA_{4+7} + BA mixture had more significant results, with greater increases in germination in *A. macroprophyllata* than in *A. purpurea*. Treatments that promoted the highest germinations were GA_{4+7} + BA at a concentration of 200 mg L^{-1} for *A. macroprophyllata* (77%) and 200 mg L^{-1} of GA_{4+7} + BA and 500 mg L^{-1} of GA_3 for *A. purpurea* (30% and 29%, respectively). Rate index, mean time and frequency of germination were distinct for both species and both treatments. Although both GA_3 and GA_{4+7} + BA promote germination, the GA_{4+7} + BA mixture was more effective than GA_3 to overcoming seed dormancy of both species, *A. purpurea* has a harder dormancy than *A. macroprophyllata*

Index terms: Annonaceae, GA_3, GA_{4+7}, N-(phenylmethyl)-aminopurine, germination-Annonaceae, *Annona diversifolia*.

[1](Trabalho 128-15). Recebido em: 11-05-2015. Aceito para publicação em: 01-03-2016. Parte de projeto realizado em pósdoc pelo primeiro autor financiado pela CAPES.

[2]Departamento de Botânica, Instituto de Biociencias de Botucatu, Universidade Estadual Paulista (UNESP), distrito de Rubião Junior, Botucatu, São Paulo, Brasil, caixa postal 510, CEP:18.608-540. E-mail: gisela@ibb.unesp.br
[3]Laboratorio de Fisiología y Química Vegetal. Instituto de Ciencias Biológicas[2], Universidad de Ciencias y Artes de Chiapas (UNICACH). Libramiento Norte Poniente 1150. Col. Lajas Maciel, C.P. 29039. Tuxtla Gutiérrez, Chiapas, México. E-mail: ivan.cruz@unicach.mx
[4]Laboratorio de Fisiología y Química Vegetal. Instituto de Ciencias Biológicas, Universidad de Ciencias y Artes de Chiapas (UNICACH). Libramiento Norte Poniente 1150. Col. Lajas Maciel, C.P. 29039. Tuxtla Gutiérrez, Chiapas, México.E mail autor corresponding: aesquinca@unicach.mx

INTRODUCTION

Plant hormones are involved in several plant mechanisms like dormancy and germination. Gibberellins (GA) are diterpenoids biosynthesized from geranyldiphosphate that present antagonistic effect with ABA to release seeds from dormancy. GAs induce the synthesis of hydrolases enzymes like α-amylases, proteases and β-glucanases (NONOGAKY et al., 2010) that act in endosperm weakening, and organ expansion during seed germination (VOEGELE et al., 2011). In addition of ABA and GA, Cytokinis (CK), plant hormones derived from adenine molecules, has been related with important role in seed germination due to antagonizes ABA and promote early seedling growth (GUAN et. al., 2014). Besides CKs are able to regulate different functions related to embryo development by affecting the cellular division, seed germination, hypocotyls and shoot growth (MIRANSARI;SMITH, 2014).

The seeds of several Annonaceae species exhibit some type of dormancy. *Annona macroprophyllata* Donn. Sm (papausa) and *Annona purpurea* Moc. & Sessé ex Dunal (chincuya) are from the low deciduous forest in Southeastern Mexico and Central America and the second species inhabits also in SouthAmerica, are edible species consumed fresh and regionally traded. Both species exhibit dormant seeds, likely reflecting an ecological adaptation given that they are dispersed at the end of the rainy season. For both species, the dormancy period varies between six and eight months (GONZÁLEZ ESQUINCA et al. 2016), (GONZALES-ESQUINCA et al.,2015).

Considering the morphological aspects of seeds of *A. macroprophyllata*, Gonzalez-Esquinca et al. (1997) in Ferreira et al. (2014) found that although the embryos are small, there is differentiation in embryonic tissues during dispersion. Furthermore, during the duration of the dormancy of *A. macroprophyllata* seeds (seven months), there were no anatomical changes. Additionally, Goméz-Castañeda et al. (2003) report that *A. purpurea* seeds have a dormancy period of more than six months of storage; after this period, only seeds treated with the gibberellin GA_3 are able to germinate.

In a study with *A. cherimola* and *A. diversifolia* seeds, Campbell and Popenoe (1968) achieved 80% germination using 350 to 500 mg.L^{-1} of GA_3. For atemoya (*A.cherimolla x A. squamosa*) cv Gefner, 778 mg L^{-1} of GA_3 promoted 80% germination, (OLIVEIRA et al., 2010). Considering the mixture of growth regulators, Da Silva et al. (2007) broke dormancy in *A. crassiflora* Mart. seeds (43% germination) using 143 mg L^{-1} of GA_{4+7}. In *Xylopia aromatica* seeds, 63 % of seedling emergence was observed using a GA_{4+7} + cytokinin N-(phenylmethyl)-aminopurine mixture (SOCOLOWSKI et al., 2011) and in atemoya, cv Gefner, 95% of germination with 329 mg L^{-1} (BRAGA et al., 2010). Therefore, considering the importance of hormones in overcoming dormancy in seeds and in germinative processes, the aim of this study was to determinate if the dormancy of *A. macrophyllata* and *A. purpurea* seeds would be able to break with the exogenous application of GA_3 and GA_{4+7} + benzyladenine (BA) mixture and the pos-maturation period reduced.

MATERIAL AND METHODS

Plant data collection

To perform the experiments, fruits of *A. macroprophyllata* Donn. Smth and *A. purpurea* Moc & Sessé ex Dunal were obtained in San Lucas, Chiapas, Mexico. Seeds were manually extracted under running water, immersed in sodium hypochlorite (3%) for 5 minutes and washed in distilled water. The seeds were then placed on filter paper for seven days on top of a laboratory bench (25 °C +/- 2 ºC) for surface drying. The initial moisture content of the seeds (10%) was determined using the oven-drying method at 105 ºC for 24 h, with four replicates of 25 seeds each (CORSATO et al., 2012). Additionally, seed viability was evaluated using the tetrazolium test with four replicates of 25 seeds each.

Evaluation of the effects of growth regulators on seed dormancy

The experimental design was a completely randomised 2 x 7 (regulators x concentrations) factorial design with 4 replicates of 25 seeds each. The growth regulators were GA_3 and GA_4+_7 + N-(phenylmethyl)-aminopurine (benzyladenine) mixture, and the concentrations: 0, 200, 400, 500, 600, 800 and 1000 mg L^{-1}.

Seeds were treated by immersion in growth regulators solutions over 96 hours under constant aeration. This period was established considering the water uptake curve previously performed (FERREIRA et al., 2014). After treatments, seeds were placed in a roll of paper moistened with distilled water (2.5 times its weight) (CORSATO et al., 2012). Seeds were kept in a germinator at 30 °C (±2 °C) average temperature at which the seeds are exposed in their habitat with a relative humidity ranging from 50 to 60% and in the absence of light (GONZALEZ-ESQUINCA et al., 2015)

Observations were made every two days

from the beginning of the experiment. The number of germinated seeds was also counted during each observation. All seeds with a primary root approximately 2 mm in length were considered to be germinated.

The following variables were calculated: percentages of germination (%G), dormant seeds (%DS) and dead seeds (%DS); relative frequency (f) according to CORSATO et al. (2012); germination rate index (GRI) according to Maguire (1962); and mean germination time (MGT) according to Edmond and Drapala (1958), cited by Ranal and Santana (2006). The GRI and MGT values were obtained using the following equations:

$$\mathbf{GRI= \Sigma G_i/T_i}$$

where GRI = daily germination rate index; G_i = daily percentage of germination; T_i = time (in days)

$$\mathbf{MGT = \Sigma G_i*T_i / \Sigma G_i}\text{s}$$

where MGT = mean time needed to achieve maximum germination; G_i = daily percentage of germination; T_i = time (in days).

The data were subjected to an analysis of variance, and the means were compared using Tukey's test at a 5% probability between regulators. Finally, a regression was performed to verify data trends in relation to concentrations. All analyses were performed using SAS (SAS Institute, Cary, NC).

RESULTS

Effects of growth regulators on total germination

Among the seeds that were soaked in water for 96 h before germination, those of *A. macroprophyllata* did not germinate. Seeds of *A. purpurea*, in turn, had a 1 to 4% germination rate (table 1).

Germination occurred when seeds received growth regulators ($p < 0.001$). There was a trend of a greater percentage of germination when regulators were used at lower concentrations. However, when comparing the effects between growth regulators, it was observed that *A. macroprophyllata* and *A. purpurea* seeds treated with GA_{4+7} + BA mixtures usually had higher percentages of germination than seeds that were treated only with GA_3 at all concentrations ($p < 0.005$). The only exception was detected in *A. purpurea* seeds: the percentage of germination with GA_{4+7} + BA was lower when 500 mg L^{-1} was used, and no differences was observed between regulators when 600 mg L^{-1} and 800 mg L^{-1} were used.

Considering the specific effects concerning GA_3 application, percentages of germination varied between 19 and 51% for *A. macroprophyllata*. The greatest effect was observed with the lower concentration (200 mg L^{-1}), whereas the least effect was detected with the application of the highest concentration (1000 mg L^{-1}). For *A. purpurea*, germination ranged from 17 to 29%, and the greatest effect was obtained with the median concentration (500 mg L^{-1}). Considering that seeds remained dormant in the absence of GA_3, the observed percentages of germination represent changes induced by GA_3 on metabolism.

The effect of the GA_{4+7} + BA mixture on *A. macroprophyllata* seeds resulted in 50 to 77% germination (with 1000 and 200 mg L^{-1}, respectively), whereas in *A. purpurea* seeds, the germination percentages varied between 25 and 30% (with 1000 mg L^{-1} and 200 mg.L^{-1}). For both species, the greatest percentages were obtained using lower concentrations ($p < 0.005$), as observed in the trend lines of both species (table 1 and figure 1).

After evaluating germination percentage, seeds that did not germinate were evaluated using a viability test (tetrazolium test), enabling the distinction between dead and dormant seeds. Therefore, only the percentage of dead seeds is presented, given that the number of dormant seeds may be calculated by subtracting the germinated and dead seeds from all evaluated seeds (table 2).

The number of dead *A. macroprophyllata* seeds was equal to the control (3-9%) when the GA_{4+7} + BA mixture was used ($p > 0.05$). Only GA_3 application at high concentrations (600 to 1000 mg L^{-1}) induced 11-15 % of dead seeds. However, for *A. purpurea*, no differences were observed between dead seeds treated with growth regulators (in any of the concentrations) and the control, suggesting that the mortality percentage reflects characteristics of this species (table 2). Therefore, growth regulator application was not the cause of seed mortality ($p > 0.05$).

In addition to their action on the germination process (modifying germination percentages), growth regulators also affected the speed at which the process occurred (figure 2), the mean time (table 3) and the frequency (figure 3).

Speed of germination of *A. macroprophyllata* seeds follows a similar trend to germination

percentage: higher speeds usually occur under lower concentrations of growth regulators. It must be highlighted that GA_{4+7} + BA, when compared to the use of GA_3, promoted the highest speeds at all concentrations. The mean time needed for germination was reduced by the use of lower concentrations of both regulators ($p < 0.05$). However, differences between GA_{4+7} + BA and GA_3 on the reduction of mean germination time were observed only with concentrations of 200, 400 and 1000 mg L^{-1} ($p < 0.05$). The speed of germination, in turn, was higher at each of the concentrations of GA_{4+7} + BA when compared to GA_3 (figure 2).

Analysis of the relative frequency of *A. macroprophyllata* germination (figure 3) also showed that germination was anticipated and more synchronised with the use of 200 mg L^{-1} to 600 mg L^{-1} of GA_3 when compared to higher concentrations. For GA_{4+7} + BA applied in concentrations up to 500 mg L^{-1}, there was a higher concentration of germinated seeds at the beginning of the period in addition to the increase in the germination percentage. Higher concentrations of GA_{4+7} + BA reduced germination percentages and induced a more dispersed germination of seeds over time.

Additionally, *A. purpurea* seeds treated with GA_{4+7} + BA germinated faster than seeds treated with GA_3. The only exception was the case of seeds treated with concentrations of 500 mg L^{-1}: there was no significant difference between regulators. Higher speeds were achieved using lower concentrations of GA_{4+7} + BA and intermediate concentrations of GA_3 (figure 3). While the use of GA_{4+7} + BA promoted the highest speeds of germination, the lowest mean times were obtained with the concentrations of 200, 400, 600 and 1000 mg.L^{-1} compared to GA_3 (Table 4). Therefore, there is no trend between this response and growth regulator concentrations.

A. purpurea seeds (figure 3) germinated more slowly and were less synchronised than *A. macroprophyllata* seeds. Additionally, *A. purpurea* seeds treated with 200 mg L^{-1} and 400 mg L^{-1} of GA_{4+7} + BA had the highest germination percentages. However, seeds treated with 400 mg L^{-1} also germinated mainly at the beginning of the period, reducing the mean time of germination.

DISCUSSION

The analysis of the findings for distinct variables of germination confirmed the dormancy of *A. macroprophyllata* and *A. purpurea* seeds. The action of growth regulators in overcoming dormancy was also corroborated like observed in other Annonaceae, atemoya (*Annona cherimolla* Mill. x *A. squamosa* L.) (BRAGA et al., 2010; OLIVEIRA et al., 2010), *A. crassiflora* (DA SILVA et al., 2007) and *X. aromatica* (SOCOLOWSKI et al., 2011).

Overall, the results suggest that in the *A. macroprophyllata* and *A. purpurea* seeds of the present study, immersion in water was insufficient to activate *de novo* GA synthesis and to antagonise ABA effects, because in this condition the seeds did not germinate. This insufficiency suggests the establishment of primary dormancy due to ABA accumulation during seed development (NAMBARA et al, 2010). Therefore, exogenous applications of growth regulators (GA_3,GA_{4+7}+BA) changed the hormonal balance and were favourable to overcome dormancy and promote germination of seeds from both species.

In this sense, although in this work the gen expression did not was evaluated, the literature is clear to report that GAs reduce the expression of ABA-responsive genes, and induce the expression of genes encoding enzymes that degrade reserves and release energy to the embryo (e.g., amylases) and that degrade the cell walls of endosperm cells, facilitating radicle protrusion (NONOGAKI et al., 2010).

The observed results of the effect of GA_3 application in *A. macroprophyllata* differ from previous findings. Campbell and Popenoe (1968) suggest that the application of GA_3 in concentrations from 350 to 500 mg L^{-1} would enhance seed germination percentages from 30 to 80% respectively. However, in the present experiment, the percentages of *A. macroprophyllata* germination of were 19% and 51% with 1000 and 200 mg L^{-1} of GA_3, respectively.

In contrast, González-Esquinca et al. (1997) cited in Ferreira et al. (2014) related that only 42% of germination was observed after the application of 10^{-3}M (346 mg L^{-1}) of GA_3 in seeds stored for 18 days and 64% with seeds stored for two months without GA_3 application. This last value is similar to the highest value observed in this study with the use of GA_3 in recently collected seeds (51%). Later, González-Esquinca et al. (2015) found that even when the embryos were completely developed at the time of dispersion, the *A. macroprophyllata* seeds needed a rest period of approximately seven months to achieve 61 to 71% germination. It is important said

that the seeds of these experiments were obtained from the same region (San Lucas city, Chiapas state, Mexico) and were germinated with the same light and temperature conditions (absence of light and 30 °C, ±2 °C) indicating that these results confirm the dormancy and necessity of plant growth regulators or storage to germinate.

As observed in this study, application of GA_{4+7} + BA in concentrations from 200 to 400 mg L^{-1} eliminates the need to wait for the rest period (6 to 8 months) to break dormancy. These regulators yielded increases in the entire germination process, from increases in germination percentage and speed to greater synchronicity of germination, favouring plant management in seedling nurseries.

Therefore, under suitable environmental conditions or when receiving stimuli through the application of growth regulators on seeds, embryos continue their development and seeds germinate. This development would not be possible with recently dispersed seeds if embryos were incomplete or undifferentiated.

For *A. purpurea*, no records were found regarding embryo development stage during seed dispersion. However, previous findings reported the high dormancy of this species, with germination of only 8% after up to six months of fruit extraction (GÓMEZ-CASTAÑEDA et al., 2003). In the present study, recently extracted seeds had 1 to 4% germination, and even with growth regulators, the highest percentages of germination were 30% (with GA_{4+7} + BA) and 29% (with GA_3). The increases in germination were 4 to 8 times greater for seeds with treatment than for seeds without treatment. Nevertheless, the germination percentage remains low for this species.

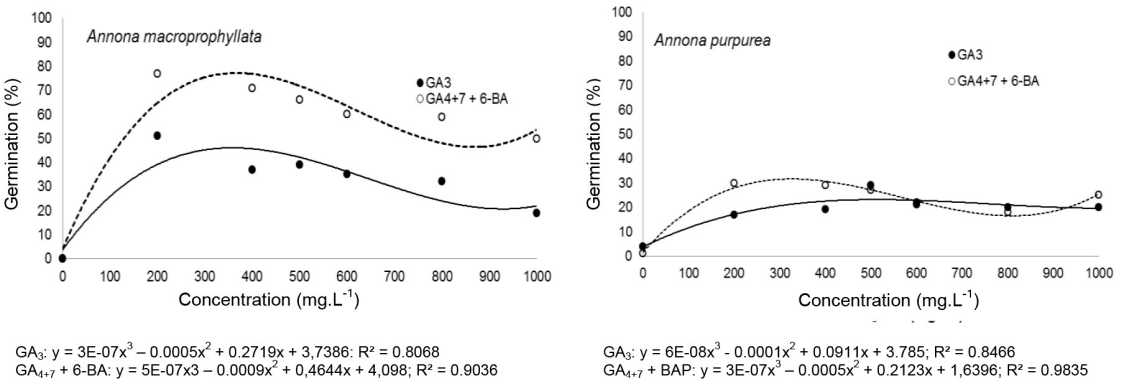
Among the regulators used in this study, the GA_{4+7} + BA mixture was more effective at overcoming dormancy of *A. macroprophyllata* and *A. purpurea* than GA_3. Using the biostimulant (GA_{4+7} + BA) leads to the synergistic effect of the two GAs and the cytokinin, enabling the use of the mixture at lower concentrations. Therefore, considering how growth regulators act, these results suggest that both GA_4 and GA_7, which are active, functioned to overcome dormancy, antagonising ABA and promoting germination (NAMBARA et al., 2010; NONOGAKY et al., 2010). Furthermore, there may have been a combined effect of cytokinin, which also acts as an antagonist to both ABA and cell division, even before radicle protrusion (MIRANSARI; SMITH, 2014; GUAN et al., 2014) and observed by Da Silva et al. (2007) in *A. crassiflora* seeds, suggesting the occurrence of events other than just cell elongation during protrusion.

Even though it promoted increases in germination, high concentrations of GA_{4+7} + BA or GA_3 reduced germination percentages, for example, with 1000 mg L^{-1} was observed the minors germinations to *A. macroprophyllata* seeds, that reduced from 51% to 19% (200 mg L^{-1} of GA_3) and from 77% to 50% (200 mg L^{-1} of GA_{4+7} + BA). This fact suggests that such a finding may be related to the control of GA biosynthesis (YAMAGUCHI, 2008; NONOGAKI et al., 2010). Therefore, high concentrations of GAs, instead of inhibiting ABA action in dormancy reduction and stimulating the synthesis of hydrolytic enzymes and/or *de novo* GA synthesis, may have acted by controlling the endogenous concentration, thus reducing their effect. The results highlight the key role of growth regulators in overcoming dormancy in *A. macroprophyllata* and *A. purpurea* seeds. In this sense, it may be considered that physiological dormancy was overcome for *A. macroprophyllata*. However, in the case of *A. purpurea*, despite the satisfactory results, more studies regarding embryo development are necessary to determine if the observed dormancy is physiological or morphophysiological.

TABLE 1- Germination (%) of *Annona macroprophyllata* and *Annona purpurea* seeds subjected to treatments with different concentrations of GA_3 e GA_{4+7} + BA

	Germination (%)			
	Annona macroprophyllata		*Annona purpurea*	
mg L^{-1}	GA_3	GA_{4+7} + BA	GA_3	GA_{4+7} + BA
0	0 Af*[1]	0 Af	4 Ac	1 Ae
200	51 Ba	77 Aa	17 Bb	30 Aa
400	37 Bbc	71 Ab	19 Bb	29 Aa
500	39 Bb	66 Ac	29 Aa	27 Bab
600	35 Bc	60 Ad	21 Ab	22 Ac
800	32 Bd	59 Ad	20 Ab	18 Ad
1000	19 Be	50 Ae	20 Bb	25 Abc

*1.The mean values followed by the same lower case letters in the columns and upper case letters in the rows, for each species, do not differ according to Tukey's test at $p<0.05$



GA_3: $y = 3E\text{-}07x^3 - 0.0005x^2 + 0.2719x + 3{,}7386$: $R^2 = 0.8068$
GA_{4+7} + 6-BA: $y = 5E\text{-}07x3 - 0.0009x^2 + 0{,}4644x + 4{,}098$; $R^2 = 0.9036$

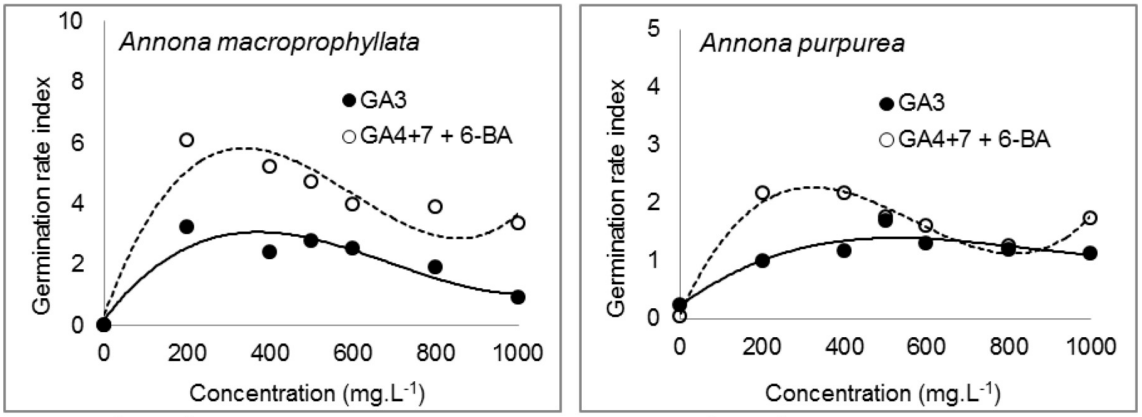
GA_3: $y = 6E\text{-}08x^3 - 0.0001x^2 + 0.0911x + 3.785$; $R^2 = 0.8466$
GA_{4+7} + BAP: $y = 3E\text{-}07x^3 - 0.0005x^2 + 0.2123x + 1{,}6396$; $R^2 = 0.9835$

FIGURE 1- Germination (%) of *Annona macroprophyllata* and *Annona purpurea* seeds subjected to treatments with different concentrations of GA_3 e GA_{4+7} + BA

TABLE 2- Dead seeds (%) of *Annona macroprophyllata* and *Annona purpurea* subjected to treatments with different concentrations of GA_3 and GA_{4+7} + BA

	Dead Seeds (%)			
	Annona macroprophyllata		*Annona purpurea*	
mg L^{-1}	GA_3	GA_{4+7} + BA	GA_3	GA_{4+7} + BA
0	1 Bb*[1]	6 Ab	4 Aa	3 Aa
200	6 Aab	3 Ab	4 Aa	6 Aa
400	16 Aa	5 Bb	6 Aa	9 Aa
500	8 Aab	9 Aab	6 Aa	8 Aa
600	11 Aab	7 Aa	7 Aa	9 Aa
800	12 Aab	6 Ab	2 Aa	7 Aa
1000	15 Aa	3 Bb	6 Aa	7 Aa

*1. The mean values followed by the same lower case letters in the columns and upper case letters in the rows, for each species, do not differ according to Tukey's test at $p<0.05$



GA_3: **y** = 2E-08x^3 - 3E-05x^2 + 0,0175x + 0.2116; R² = 0.8585
GA_{4+7} + BAP: **y** = 4E-08x^3 - 8E-05x^2 + 0.0374x + 0.3416; R² = 0.8822.

GA_3: **y** = 3E-09x^3 - 7E-06x^2 + 0.0053x + 0.2264; R² = 0.8881
GA_{4+7} + BAP: **y** = 2E-08x^3 - 3E-05x^2 + 0.0155x + 0.0975; R² = 0.9784

FIGURE 2- Germination rate index of *Annona macroprophyllata* and *Annona purpurea* seeds subjected to treatments with different concentrations of GA_3 and GA_{4+7} + BA

TABLE 3-Mean germination time of *Annona macroprophyllata* and *Annona purpurea* seeds subjected to treatments with different concentrations of GA_3 and GA_{4+7} + BA

	Mean germination time			
	Annona macroprophyllata		*Annona purpurea*	
mg L^{-1}	GA_3	GA_{4+7} + BA	GA_3	GA_{4+7} + BA
0	0 Ad*[1]	0 Ac	8.62 Ab	5.25 Ab
200	17.99 Abc	13.16 Bc	18.72 Aa	14.54 Bab
400	16.29 Abc	14.55 Bab	16.56 Aab	13.61 Bab
500	14.85 Ac	14.72 Aa	18.26 Aab	15.76 Aa
600	14.42 Ac	16.42 Aa	17.51 Aa	13.74 Bab
800	18.13 Ab	15.99 Aa	18.44 Aa	15.51 Aa
1000	22.45 Aa	15.94 Ba	18.94 Aa	14.82 Bab

*1. The mean values followed by the same lower case letters in the columns and upper case letters in the rows, for each species, do not differ according to Tukey's test at $p<0.05$

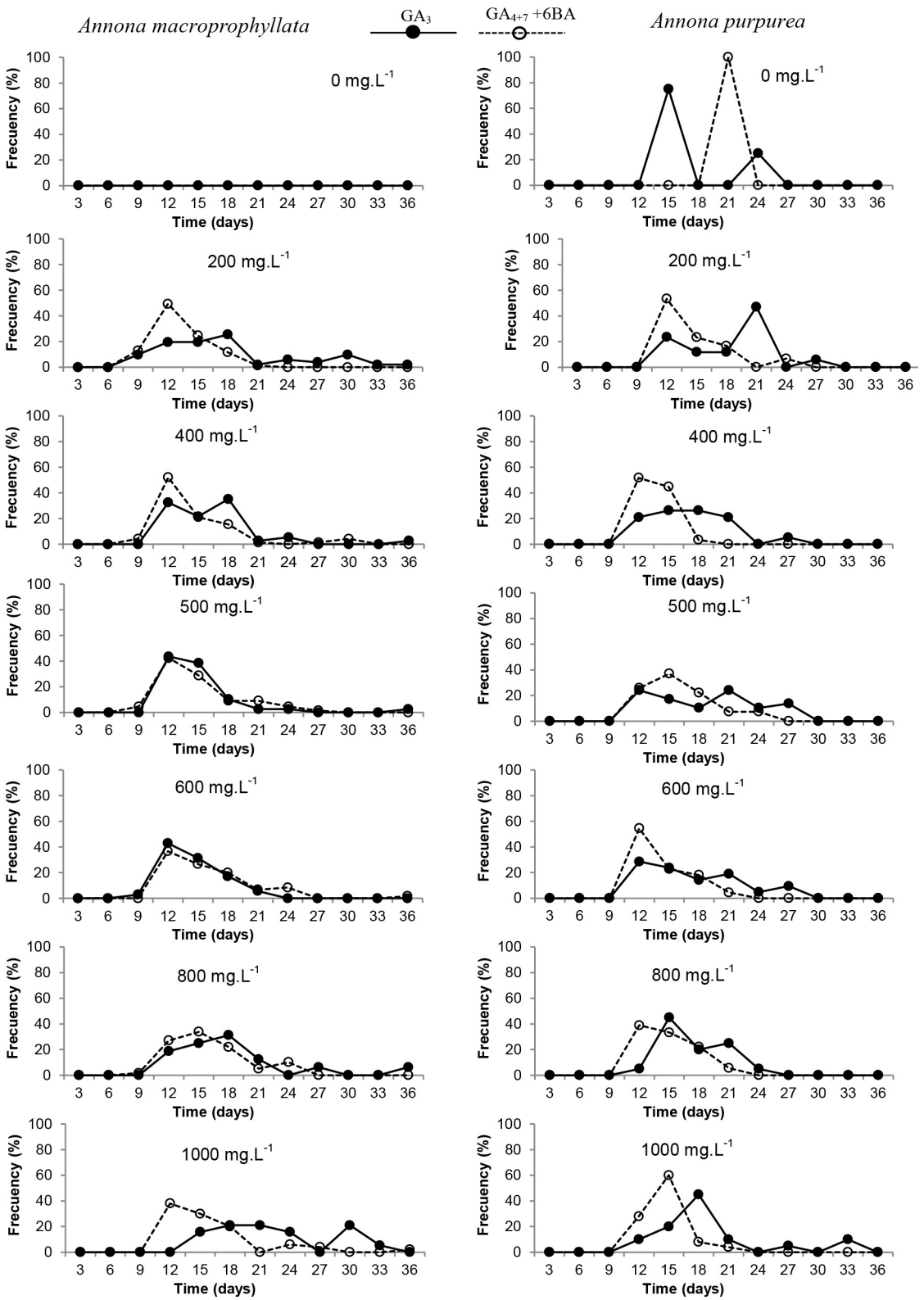


FIGURE 3- Relative germination frequency (%) of *Annona macroprophyllata* and *Annona purpurea* seeds subjected to treatments with different concentrations of GA_3 and GA_{4+7} + BA

CONCLUSION

Therefore the GA_{4+7} + BA mixture was more effective than GA_3 to overcoming seed dormancy of both species. Besides, *A. purpurea* has a harder dormancy than *A. macroprophyllata.*

ACKNOWLEDGEMENTS

The first author thanks to the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) for providing schoolarship posdoctorate and also thanks to the *Universidad de Ciencias y Artes de Chiapas* (UNICACH) for the opportunity to make the postdoc.

REFERENCES

BRAGA, J.F.; FERREIRA, G.; PINHO, S.Z.; BRAGA, L.F.; SOUSA, M.P. Germination of atemoya (*Annona cherimola* Mill. x *A. squamosa* L.) CV. Gefner seeds subjected to treatments with plant growth regulators. **International Journal of Science and Nature**, Daca, v.1, n. 2, p. 120-126, 2010.

CAMPBELL, C.W.; POPENOE, J. Effect of gibberellic acid on seed dormancy of *Annona diversifolia* Saff. **Tropical Region American Society for Horticultural Service**, Mount Vermon, v. 11, p. 31-36, 1968.

CORSATO, J. M.; FERREIRA, G.; BARBEDO, C. J. Desiccation tolerance in seeds of *Annona emarginata* (Schldtl.) H. Rainer and action of plant growth regulators on germination. **Brazilian Journal of Plant Physiology**, Campos dos Goytacazes, v.24, n.4, p.253-260, 2012.

DA SILVA, E. A.; DE MELO, D. L.; DAVIDE, A. C.; DE BODE, N.; ABREU, G. B.; FARIA, J. M.; HILHORST, H. W. Germination ecophysiology of *Annona crassiflora* seeds. **Annals of Botany**, London, v. 99, n.5, p. 823-830, 2007.

FERREIRA, G.; GONZÁLEZ-ESQUINCA, A.R.; DE-LA-CRUZ-CHACON, I. Water uptake by *Annona diversifolia* Saff. and *A. purpurea* Moc. & Sessé ex Dunal seeds (Annonaceae). **Revista Brasileira de Fruticultura**, Jaboticabal, v. 36, p. 288-295, 2014. Número Especial

GOMEZ-CASTAÑEDA, J.A.; RAMÍREZ, H.; BENAVIDES-MENDOZA, A.; ENCINA-RODRIGUEZ, I. Germination and seedling development of soncoya (*Annona purpurea* Moc y Sessé) in relation to gibberelins and abscisic levels. **Revista Chapingo Serie Horticultura,** Texcoco, v. 9, n.2, p.243-253, 2003.

GONZÁLEZ-ESQUINCA, A.R.; DE-LA-CRUZ-CHACÓN, I.; DOMÍNGUEZ-GUTU, L.M.. Dormancy and germination of *Annona macroprophyllata* Donn. Sm (Annonaceae): the importance of micropylar plug and seed position. **Botanical Sciences,** Ciudad de México, v.93, n.3, p.1-7, 2015.

GONZÁLEZ-ESQUINCA, A.R.; DE-LA-CRUZ-CHACÓN I.; CASTRO-MORENO, M.; RILEY-SALDAÑA C.A. Phenological strategies of Annona from deciduous forest of Chiapas, Mexico. **Botanical Sciences**. Ciudad de México, v. 94, n. 3, p. 531-541. 2016.

GUAN, C., WANG, X., FENG, J., HONG, S., LIANG, Y., REN, B., ZUO, J.Cytokinin antagonizes abscisic acid-mediated inhibition of cotyledon greening by promotingthe degradation of ABSCISIC ACID INSENSITIVES protein Arabidopsis. **Plant Physiology,** Rockville, v. 164, p.1515-1526, 2014.

MIRANSARI, M.; SMITH, D.L. Plant hormones and seed germination. **Environmental and Experimental Botany**, Oxford, v.99, p.110-121, 2014.

NAMBARA, E.; OKAMOTO, M.; TATEMATSU, K.; YANO, R.; SEO, M.. Abscisic acid and the control of seed dormancy and germination. **Seed Science Research**, Cambridge, v. 20, p.55-67, 2010.

NONOGAKI, H.; BASSEL,G.W.; BEWLEY, J.D. Germination – still a mystery. **Plant Science**, Limerick, v. 179, p.574-581, 2010.

OLIVEIRA, M. C. D.; FERREIRA, G.; GUIMARÃEs, V. F.; DIAS, G. B. Germination of atemoya seeds (*Annona cherimola* Mill. x *A. squamosa* L.) cv 'Gefner' subjected to treatments with Gibberellic acid (GA3) and ethephon. **Revista Brasileira de Fruticultura**, Jaboticabal, v. 32, n.2, p. 544-554, 2010.

SOKOLOWSKI, F.; CICERO, S.M. Use of growth regulators to overcome seed dormancy in *Xylopia aromatica* (Annonaceae). **Seed Science and Technology**, Zürich, v.39, n.1, p.21-28, 2011.

VOEGELE, A., LINKIES, A., MULLER, K., LEUBNER-METZGER, G. Members of the gibberellin receptor gene family GID1 (Gibberellin Insensitive Dwarf1) playdistinct roles during *Lepidium sativum* and *Arabidopsis thaliana* seed germina-tion. **Journal of Experimental Botany,** Oxford, 155, 1851–1870, 2011.

YAMAGUCHI, S. Gibberellin metabolism and its regulation. **Annual Review of Plant Biology**, Palo Alto, v. 59, p. 225-251, 2008.