SOLUBLE SUGARS AND GERMINATION OF *Annona emarginata* (Schltdl.) H. Rainer SEEDS SUBMITTED TO IMMERSION IN GA_3 UP TO DIFFERENT WATER CONTENTS[1]

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ABSTRACT – The objective of this study was to evaluate the effect of different water contents achieved by *Annona emarginata* (Schltdl.) H. Rainer seeds during immersion in GA_3 solutions, in variation of soluble sugars levels and germination. Seeds with 10% of initial water content were submitted to imbibition in GA_3 solutions with concentrations of 0; 250; 500; 750 and 1000 mg L^{-1} and when they reached the water content of 15%, 20%, 25%, 30% and 35%, the quantification of soluble sugars levels and germination test were performed. Seeds immersed up to they reach 15% of water with GA_3 and immersed up to the water acquisition of 20% without GA_3, presented higher soluble sugars levels and germination percentage, which were decreased when the seeds reached 30% and 35% of water, independently of the presence of the plant growth regulator. It was conclude that different water contents reached by the seeds in immersion treatments with GA_3 affect the soluble sugars levels and germination percentage of *Annona emarginata* seeds. Thus, in treatments with *Annona emarginata*, the seeds must remain immersed in water without GA_3 up to they reach 20% of water, as higher water contents (35%) reduce the soluble sugars levels and the seed germination percentage.
Index terms: Annonaceae, “araticum-de-terra-fria”, carbohydrates, imbibition.

AÇÚCARES SOLÚVEIS E GERMINAÇÃO DE SEMENTES DE *Annona emarginata* (Schltdl.) H. Rainer SUBMETIDAS À IMERSÃO EM GA_3 ATÉ DIFERENTES TEORES DE ÁGUA

RESUMO – O objetivo deste trabalho foi avaliar o efeito de diferentes teores de água atingidos pelas sementes de *Annona emarginata* (Schltdl.) H. Rainer durante a imersão em soluções de GA_3, na variação dos teores de açúcares solúveis e na germinação. Sementes com teor de água inicial de 10% foram colocadas para embeber em soluções com as concentrações de 0; 250; 500; 750 e 1.000 mg L^{-1} de GA_3, e ao atingirem os teores de 15%, 20%, 25%, 30% e 35% de água, procedeu-se à quantificação dos teores de açúcares solúveis e ao teste de germinação. As sementes imersas até atingirem 15% de água com a adição de GA_3 e imersas até a aquisição de 20% de água sem a adição de GA_3 apresentaram maiores teores de açúcares solúveis e porcentagens de germinação, os quais foram reduzidos à medida que as sementes atingiram 30% e 35% de água, independentemente do uso do regulador vegetal. Conclui-se que os diferentes teores de água alcançados pelas sementes nos tratamentos de imersão afetam os teores de açúcares solúveis e a germinação de sementes de *Annona emarginata*. Assim, em tratamentos com *Annona emarginata*, as sementes devem permancer imersas na água sem a adição de GA_3 até a aquisição de 20% de água, uma vez que elevados teores de água (35%) reduzem os teores de açúcares solúveis totais e a porcentagem de germinação das sementes.
Termos para indexação: Annonaceae, “araticum-de-terra-fria”, carboidratos, embebição.

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INTRODUCTION

The germination process starts with water uptake by the seed, leading to a number of metabolic changes which culminate in radicle protrusion (NONOGAKI et al., 2010). Thus, determination of water acquisition curve helps setting times for immersion treatments of seeds in aqueous solutions, as performed with plant growth regulators (SOCOLOWSKI; CICERO, 2011) or priming (VARIER et al., 2010), where the duration of treatments has been defined according to the duration of Phase I.

In studies with plant growth regulators in Annonaceae, the immersion period of the seeds is generally defined according to the stabilization of the water acquisition curve (FERREIRA et al., 2006; COSTA et al., 2011), when the transition from Phase I to Phase II of the germination process occurs, considering that the metabolic processes has already initiated in Phase I and that, in Phase II, the little water uptake by seeds occurs due to some change in the osmotic potential, caused by the degradation of reserves.

In this context, Braga et al. (2010) used to soak the seeds in plant growth regulators (GA_3, GA_{4+7} + N-(phenylmethyl)-1H-purine-6-amine and CK+GA+AX) for 36 hours (transition from Phase I to Phase II) in order to overcome the dormancy of atemoya seeds (*Annona cherimola* Mill. x *A. squamosa* L.) reaching up to 95.45% germination. In *Annona emarginata* (Schltdl.) H. Rainer seeds, Costa et al. (2011) and Corsato et al. (2012) used the immersion for 60 hours (based on transition from Phase I to Phase II) in GA_{4+7} + N-(phenylmethyl)-aminopurine, obtaining 27% and 70% germination, respectively.

Considering the activation of different metabolic processes with the water uptake by the seed, it is possible to observe an increase in respiration and the beginning of consumption of sugars, minutes after the start of imbibition (TONINI et al., 2010). Besides acting as a substrate for respiration, sugars such as sucrose and glucose are carbon sources for metabolites production including amino acids, lipids, proteins and complex carbohydrates such as starch and cellulose (ROLLAND et al., 2006).

The objective of this study was to evaluate the effect of different water contents achieved by *Annona emarginata* (Schltdl.) H. Rainer seeds during immersion in GA_3 solutions, in the variation of soluble sugars levels and germination.

MATERIAL AND METHODS

Water acquisition curve

After extraction, the seeds were submitted to artificial drying at 32ºC, being monitored up to they reach 10% of water content. To verify the water acquisition pattern during the seed germination phases, 4 replicates of 25 seeds immersed in deionized water were used, under constant temperature of 25±2°C, in the absence of light, with artificial aeration system. Periodical weights for the calculation of seed water uptake were performed and by the stabilization of the water intake, the seeds were removed from the water and placed in 'germitest' paper, with the weighing being held until the beginning of phase III, when the emission of the primary root occurs. To study the variation in the seed water content, in phase I and the beginning of phase II, the Brody model, or monomolecular model, was adjusted to the equation $y=\alpha\ [1-\beta \exp(-c\ x)]$, with y = moisture degree (%), x = time, α, β e c = model parameter (MISCHAN et al., 2011).

Germination test

Four sampling points were determined in phase I of the germination process (15%, 20%, 25% and 30% water content) from the water acquisition curve and another sampling point on the curve stabilization, corresponding to the transition point from phase I to phase II (35% water).

The *A. emarginata* seeds with initial water content of 10% were submitted to immersion in GA_3 solutions at 0, 250, 500, 750 and 1000 mg L^{-1} until they reach 15%, 20%, 25%, 30% and 35% of water content, under constant temperature of 25±2°C, in the absence of light and with artificial aeration system (aquarium pump). The experimental design was completely randomized in 5x5 factorial (GA_3 concentrations x water content), totalizing 25 treatments, with 4 replicates of 20 seeds.

When the seeds reached the correspondent water contents, they were removed from the solutions and submitted to the germination test, carried out on germitest paper, moistened with deionized water and held in a germination chamber under alternated photoperiod and temperature (8 hours of dark at 20°C and 16 hours of light at 30°C) (COSTA et al., 2011). The germinated seeds were counted each 2 days, considering as being germinated, the seeds with at least 2mm of primary root.

Total Soluble Sugars

As the seeds reached 15%, 20%, 25%, 30% and 35% of water, 15 seeds per treatment were immersed in liquid nitrogen and stored in ultrafreezer (-80°C) for soluble sugars quantification. The soluble sugars extraction was performed according to an adapted methodology from Garcia et al. (2006), using three replicates with 100mg of grounded seeds, per treatment. The total soluble sugars were estimated colorimetrically by phenol-sulfuric method (DUBOIS et al., 1956), using glucose (100µg mL^{-1}) as a standard and they were expressed as milligrams per gram of dry mass (mg g^{-1} DM).

The data of germination test and soluble sugars were submitted to homogeneity test (Levene's test), to variance analysis (F test) and the average data were compared by Tukey test at 5% of significance.

RESULTS AND DISCUSSION

With the water acquisition curve it was possible to characterize Phases I, II and III of germination of *A. emarginata* seeds, in accordance with the triphasic pattern proposed by Bewley and Black in 1994 (FERREIRA et al., 2006) (Figure 1). Phase I lasted 109 hours, from the start of imbibition, finishing when the seeds reached 35.2% of water, as described for endospermatic seeds (CARVALHO; NAKAGAWA, 2000). Phase II begins when the curve shows a tendecy for stabilization, lasting an average of 227 hours, reaching Phase III (visible germination) with approximately 336 hours (14 days) from the onset of imbibition, when the seeds had 40% of water.

Phase I is reported as rapid and it may vary depending on the nature and composition of the seed coat and seed reserve, as well on the environment in which it is situated (SORIANO et al., 2011), even in seeds from the same botanical family, as the Annonaceae. Therefore, atemoya seeds presented transition between Phases I and II after 36 hours from the onset of imbibition (FERREIRA et al., 2006), while in *A. emarginata* seeds the process is even slower, approximately 109 hours to reach Phase II.

From the water acquisition curve it was possible to determine the time required for the seeds of *A. emarginata* to reach water contents of 15% (3 hours), 20% (8 hours), 25% (18 hours), 30% (42 hours) and 35% (98 hours). As seen in water acquisition curve, having all these water contents, the seeds were in Phase I of the germination curve (Figure 1). Table 1 shows that the soluble sugars levels and the germination percentage have been affected by the water contents reached by seeds during immersion, presenting significant interaction between the water content and the concentration of GA_3 for both soluble sugars contents and for germination percentage.

It can be seen in Table 2 that seeds immersed without plant growth regulator up to they reach 15% of water, with subsequent transfer to germination paper, presented lower soluble sugars levels (27 mg g^{-1} DM) than seeds immersed up to they reach 20% of water (43 mg g^{-1} DM), the same has been observed in relation to germination percentage (71% and 90%, respectively). In this context, it is known that when seeds begin imbibition, there is an increase in the respiratory activity, which may have been responsible for higher germination percentages (TONINI et al., 2010).

So that seeds immersed up to they reach 15% of water could germinate, it was necessary to add GA_3, being the concentrations of 250 and 1000 mg L^{-1}, responsible for a 20% increase in germination percentage (91% and 90%, respectively) (Table 2). It can also be checked that the use of 1000 mg L^{-1} GA_3 resulted in higher levels of soluble sugars (Table 3). The results suggest that *A. emarginata* seeds can activate the germination process when they reach 20% of water without the use of GA_3, which for seeds immersed up to they reach 15% of water was only possible when supplied with the regulator.

The action of the gibberellins is reported in several studies of germination in Annonaceae species, such as *Xylopia aromatica* (Lam.) Mart. (SOCOLOWSKI; CICERO, 2011), *Annona squamosa* L. (FERREIRA et al., 2002; SOUSA et al., 2008), *Annona crassiflora* Mart. (BERNARDES et al., 2007). Braga et al. (2010), working with *A. cherimola* x *A. squamosa* seeds, had the highest germination percentage (95.45%) when plant growth regulators were used, compared to 52% germination of the control treatment. In these studies, however, only the time that seeds had been kept immersed in solutions with plant growth regulators had been reported but no information were available about the water content that seeds reached.

The effect of gibberellin is based on the gene expression related to the synthesis of hydrolases responsible for the degradation of the endosperm reserves, with the release of simpler compounds, used as energy source for the embryo growth (RAJJOU et al., 2012). Besides, gibberellin has an important role in the activation and/or synthesis of enzymes that promote the weakening of the tissues which surround the embryo, facilitating the radicle protrusion (OGAWA et al., 2003). Thus, in this study, the highest percentages of germination have been obtained with the addition of exogenous GA_3 to the immersed seeds up until they reach 15% of water, or naturally, by immersing the seeds up until the acquisition of the adequate water content.

When *A. emarginata* seeds were kept immersed up to they reach 35% of water (without regulator) the percentage of germination did not differ from the seeds kept immersed up to they reach 15% of water without plant growth regulator (71% and 64%, respectively). However, when the seeds were immersed up to they reach 35% of water with the regulator, there was no increase in the germination percentage, as well as with 15% of water, showing the lowest germination percentage at each regulator concentration (Table 2).

These results demonstrate that keeping seeds up to transition from Phase I to Phase II, when the seeds reach 35% of water, has been harmful for the germination process, resulting in the lowest levels of soluble sugars and the lowest germination percentages, results that GA_3 has not reversed. Costa et al. (2011) have also observed low germination percentage (27%) when the seeds of *A. emarginata* were kept immersed in solution for 60 hours, up to the transition from Phase I to Phase II, when the seeds reached the water content of 28.35%.

Therefore, the longer offered for the seeds to achieve the Phases shift, 98 hours in this paper and 60 hours for Costa et al. (2011), may have led to a smaller availability of oxygen or an increased need of oxygen for respiration, even with artificial aeration system constantly providing oxygen. Thus, the increase in mitochondrial activity during the germination process may have resulted in higher oxygen consumption (BENAMAR et al., 2003).

In case the oxygen supply has not been adequate for the seeds, the level of ABA may have been increased, resulting in lower germination percentages due to the antagonism with gibberellin. Furthermore, when seeds are subjected to hypoxia, an increase in sensibility to ABA is observed (BENECH-ARNOLD et al., 2006). However, such hypotheses on the reduction of germination percentage when seeds remain submerged up to they reach 35% of water should still be tested.

TABLE 1- Variance analysis for total soluble sugars (mg g^{-1} DM) and germination percentage (%) of *Annona emarginata* (Schltdl.) H. Rainer seeds submitted to immersion with GA_3 concentrations, up until they reach different water contents.

Variation factors	Total Soluble Sugars	Germination (%)
Water content	15.3**	117.7**
GA_3 concentration	4.4*	1.4^{ns}
Water content x GA_3 concentration	6.5**	6.6**
CV (%)	10.15	7.65

F test. *significant at 5% of probability; **significant at 1% of probability; ^{ns}no significant.

TABLE 2 - Germination (%) of *Annona emarginata* (Schltdl.) H. Rainer seeds submitted to immersion with GA_3 concentrations, up to they reach different water contents.

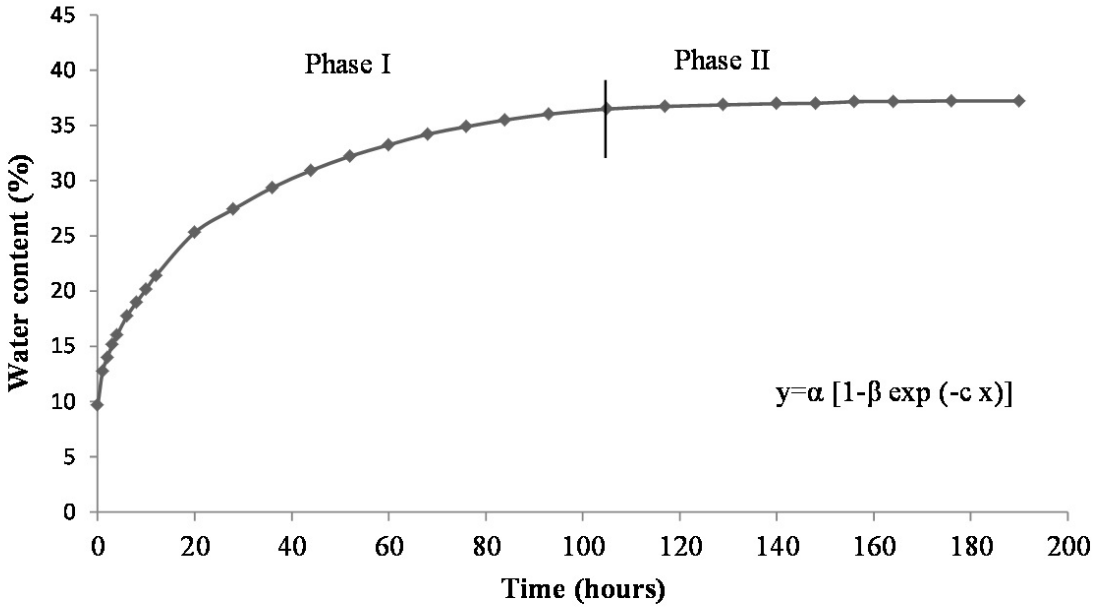
Water content (%)	GA_3 concentrations (mg L^{-1})				
	0	250	500	750	1000
15	71 Bb	91 Aa	86 ABab	87 ABab	90 Aa
20	90 Aa	91 Aa	92 Aa	95 Aa	84 Aa
25	79 Aab	90 Aa	84 Aab	75 Ab	91 Aa
30	76 Aab	56 Bb	70 ABbc	70 ABb	64 ABb
35	64 Ab	51 Ab	65 Ac	51 Ac	51 Ab

Averages followed by the same letter, lowercase in columns and uppercase in lines, do not differ significantly by Tukey test at 5% of probability.

TABLE 3- Total soluble sugars (mg g^{-1} DM) in *Annona emarginata* (Schltdl.) H. Rainer seeds submitted to immersion with GA_3 concentrations, up to they reach different water contents.

Water content (%)	GA_3 concentration (mg L^{-1})				
	0	250	500	750	1000
15	27 Cb	34 ABCab	29 BCb	37 ABa	41 Aa
20	43 Aa	33 Bab	38 ABa	34 Ba	31 Bb
25	35 ABb	31 Bab	34ABab	39 ABa	40 Aa
30	31 ABb	38 Aa	31 ABab	24 Bb	34 Aab
35	29 ABb	29 ABb	27 ABb	22 Bb	32 Ab

Averages followed by the same letter, lowercase in columns and uppercase in lines, do not differ significantly by Tukey test at 5% of probability.



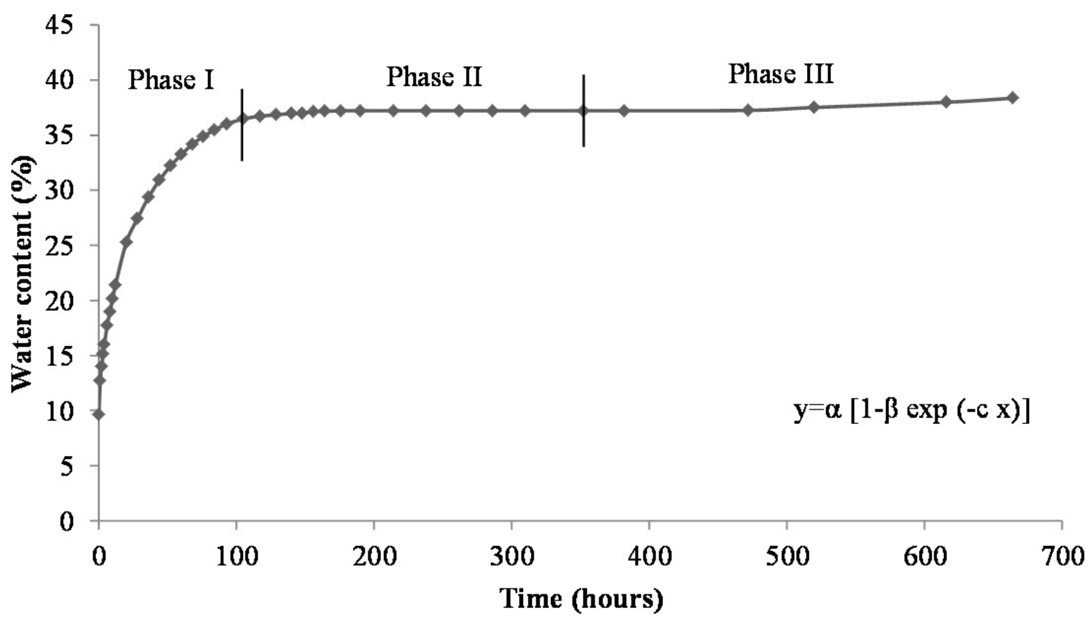


FIGURE 1- Water acquisition curve of *Annona emarginata* (Schltdl.) H. Rainer seeds. **A** Phases I and II. **B** Phases I, II and III.

CONCLUSION

The different water contents reached by the seeds in the immersion treatments with GA_3 affect the soluble sugars levels and the germination percentage of *Annona emarginata* seeds. Thus, in treatments with *Annona emarginata*, the seeds must remain immersed in water without GA_3 up to they reach 20% of water, as higher water contents (35%) reduce soluble sugars levels and seed germination percentage.

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REFERENCES

BENAMAR, A.; TALLON, C.; MACHEREL, D. Membrane integrity and oxidative properties of mitochondria isolated from imbibing pea seeds after priming or accelerated ageing. **Seed Science Research**, Wallingford, v.13, p.35-45, 2003.

BENECH-ARNOLD, R.L.; GUALANO, N.; LEYMARIE, J.; CÔME, D.; CORBINEAU, F. Hypoxia interferes with ABA metabolism and increases ABA sensitivity in embryos of dormant barley grains. **Journal of Experimental Botany**, Oxford, v.57, n.6, p.1423-1430, 2006.

BERNARDES, T.G.; ESTRELA, C.T.; NAVES, R.V.; REZENDE, C.F.A.; MESQUITA, M.A.M.; PIRES, L.L. Efeito do armazenamento e de fitormônios na qualidade fisiológica de sementes de araticum (*Annona crassiflora* Mart.). **Pesquisa Agropecuária Tropical**, Goiânia, v.37, n.3, p.163-168, 2007.

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.

CARVALHO, N.M.; NAKAGAWA, J. **Sementes:** ciência, tecnologia e produção. Jaboticabal: Funep, 2000. 588p.

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

COSTA, P.N.; BUENO, S.S.C.; FERREIRA, G. Fases da germinação de sementes de *Annona emarginata* (Schltdl.) H.Rainer em diferentes temperaturas. **Revista Brasileira de Fruticultura**, Jaboticabal, v.33, n.1, p.253-260, 2011.

DUBOIS, M.; GILLES, K.A.; HAMILTON, J.K.; REBERS, P.A.; SMITH, F. Colorimetric method for determination of sugars and related substances. **Analytical Chemistry**, Washington, v.28, n.3, p.350-356, 1956.

FERREIRA, G.; ERIG, P.R.; MORO, E. Produção do porta-enxerto (*Annona squamosa* L.) com o uso de reguladores vegetais. **Revista Brasileira de Fruticultura**, Jaboticabal, v.24, n.3, p.637-640, 2002.

FERREIRA, G.; GUIMARÃES, V.F.; PINHO, S.Z.; OLIVEIRA, M.C.; RICHART, A.; BRAGA, J.F.; DIAS, G.B. Curva de absorção de água em sementes de atemoia (*Annona cherimola* Mill. x *Annona squamosa* L.) cv. 'Gefner'. **Revista Brasileira de Fruticultura**, Jaboticabal, v.28, n.1, p.121-124, 2006.

GARCIA, I.S.; SOUZA, A.; BARBEDO, C.J.; DIETRICH, S.M.C.; FIGUEIREDO-RIBEIRO, R.C.L. Changes in soluble carbohydrates during storage of *Caesalpinia echinata* Lam. (Brazilwood) seeds, na endangered leguminous tree from the brazilian atlantic forest. **Brazilian Journal Biology**, São Carlos, v.66, n.2B, p.739-745, 2006.

MISCHAN, M.M.; PINHO, S.Z.; CARVALHO, L.R. Determination of a point sufficiently close to the asymptote in nonlinear growth functions. **Scientia Agricola**, Piracicaba, v.68, n.1, p.109-114, 2011.

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

OGAWA, M.; HANADA, A.; YAMAUCHI, Y.; KUWAHARA, A.; KAMIYA, Y.; YAMAGUCHI, S. Gibberellin biosynthesis and response during Arabidopsis seed germination. **The Plant Cell**, Rockville, v.15, p.1.591-1.604, 2003.

RAJJOU, L.; DUVAL, M.; GALLARDO, K.; CATUSSE, J.; BALLY, J.; JOB, C.; JOB, D. Seed germination and vigor. **Annual Review Plant Biology**, Palo Alto, v.63, p.507-533, 2012.

ROLLAND, F.; BAENA-GONZALEZ, E.; SHEEN, J. Sugar sensing and signaling in plants: conserved and novel mechanisms. **Annual Review Plant Biology**, Palo Alto, v.57, p.675-709, 2006.

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

SORIANO, D.; OROZCO-SEGOVIA, A.; MÁRQUEZ-GUZMÁN, J.; KITAJIMA, K.; BUEN, A.G.; HUANTE, P. Seed reserve composition in 19 tree species of a tropical deciduous forest in Mexico and its relationship to seed germination and seedling growth. **Annals of Botany**, London, v.107, p.939-951, 2011.

SOUSA, S.A.; DANTAS, A.C.V.L.; PELACANI, C.R.; VIEIRA, E.L.; LEDO, C.A.S. Superação da dormência em sementes de pinha. **Caatinga**, Mossoró, v.21, n.4, p.118-121, 2008.

TONINI, P.P.; PURGATTO, E.; BUCKERIDGE, M.S. Effects of abscisic acid, ethylene and sugars on the mobilization of storage proteins and carbohydrates in seeds of the tropical tree *Sesbania virgata* (Leguminosae). **Annals of Botany**, London, v.106, p.607-616, 2010.

VARIER, A.; VARI, A.K.; DADLANI, M. The subcellular basis of seed priming. **Current Science**, Bangalore, v.99, n.4, p450-456, 2010.