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Osmopriming duration in Araçá-boi seeds germination

Duração do osmocondicionamento na germinação de sementes de araçá-boi

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Abstract: The Araca-boi is a native fruit of the Amazon region and its fruits have great potential for agro-industrial use due to the intrinsic characteristics of the pulp. The seeds are dormant and the primary cause could be due to the presence of inhibiting chemicals germination and can also be caused by the impermeability in tegument. The objective of this study was to assess the osmopriming efficiency promoted by Sucrose ($C_{12}H_{22}O_{11}$) and Potassium Nitrate (KNO_3) on germination of seeds of araca-boi in different immersion times. Thus, the seeds were osmoconditioned in following solutions: $C_{12}H_{22}O_{11}$, KNO_3, KNO_3 + $C_{12}H_{22}O_{11}$ and H_2O, by period of 6, 12 and 24 h. Throughout of 70 days were evaluated the germination percentage and germination rate (index). The experiment is based on a completely randomized in a factorial analysis 4x3 with four replications of 10 seeds. The osmopriming of Araca-boi seed in KNO_3 solution was shown to be efficient both to promote germination and the germination rate. The Araca-boi seeds osmoconditioned in KNO_3 solution has an average efficiency of 99% in the germination percentage up to 70 days. The osmopriming period showed no influence on seed germination of the seeds of Araca-boi.

Resumo: O araçá-boi é uma frutífera nativa da região Amazônica e seus frutos apresentam grande potencial de aproveitamento agroindustrial devido às características intrínsecas de sua polpa. Suas sementes são dormentes e a principal causa pode ser devido à presença de substâncias químicas inibidoras da germinação e também pode ser causada pela impermeabilidade do tegumento. Neste contexto, o objetivo neste trabalho foi avaliar a eficiência do osmocondicionamento promovido por Sacarose ($C_{12}H_{22}O_{11}$) e Nitrato de Potássio (KNO_3) na germinação de sementes de araçá-boi, em diferentes tempos de imersão. Para tal, as sementes foram osmocondicionadas em soluções de $C_{12}H_{22}O_{11}$, KNO_3, KNO_3,+ $C_{12}H_{22}O_{11}$ e H_2O, por períodos de 6, 12 e 24 h. Foram avaliadas no período de 70 dias, a porcentagem de germinação e a velocidade de germinação (índice). O delineamento utilizado foi o inteiramente casualizado em fatorial 4x3, com 4 repetições de 10 sementes. O osmocondicionamento de sementes de araçá-boi em solução de KNO_3 é eficiente para promover a germinação e a velocidade de germinação. Sementes de araçá-boi osmocondicionadas em KNO_3 apresentam porcentagem de germinação de 99% até os 70 dias. O período de osmocondicionamento não influencia na germinação das sementes de araçá-boi.

Palavras-chave: *Eugenia stipitata*. Dormência. Priming.

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INTRODUCTION

Araçá-boi (*Eugenia stipitata* McVaugh) is a native fruit specie from Amazon jungle, which belongs to the Myrtaceae family, and it is widely distributed in the Western Amazon, and is usually grown on a small scale in Peru, Bolivia, Ecuador, Colombia and Brazil (CHÁVES FLORES and CLEMENT, 1984). The fruits have a great potential for agro-industrial use due to the good chemical characteristics and to sensory attributes that determine acceptability (ROGEZ *et al*., 2004).

The seeds germination are hypogeal, cryptocotylar and usually occurs at 50 to 280 days from the beginning to the end of the germination process (ANJOS and FERRAZ, 1999). Some studies presented that seeds dormancy are one of the major cause of long germination period, as well as the slowness in the seedling emergence of this specie. Seeds dormancy may occur due to the presence of germination inhibitors, such as abscisic acid and phenolic compounds (SERT *et al*., 2009). According to Pineto *et al*. (1981), the seeds dormancy may also be caused by seed husk impermeability . Moreover, Gentil and Ferreira (1999) confirmed that araçá-boi seed husks present mechanical restriction of embryo expansion. According to literature, araçá-boi seeds dormancy caused by germination inhibitors and seed husk impermeability can be removed by leaching and the partial removal of the seed husk on the side of the root protrusion, respectively (MENDES, 2011; SERT *et al*., 2009). Other inductive methods for overcoming seed dormancy may be used, such as osmopriming in organic and inorganic substances, aiming at the formation of a greater number of viable and uniform seedlings.

Osmopriming consists in the controlled hydration of seeds in an osmotic solution, stimulating the metabolism without the emission of the primary root (NASCIMENTO, 2004). This conditioning process activates degradation, translocation, and assimilation of reserves, allowing the seeds to achieve relatively uniform metabolic status by interrupting the water supply. Several studies have shown that osmopriming increase the seeds germination, allowing for more rapid and uniform emergence of seedling (NASCIMENTO; ARAGÃO, 2004; PEREIRA *et al*., 2008; ARAÚJO *et al*., 2011). Kissmann *et al*. (2011), used inorganic osmotic agents, such as potassium nitrate (KNO_3), and organic osmotic agents, such as sucrose ($C_{12}H_{22}O_{11}$), water (H_2O), among others to promote osmopriming on *Jacaranda decurrens* subsp. *symmetrifoliolata* seedlings. Besides that, the index of germination rate may not be influenced by pre-imbibition, which is a treatment that does not necessarily influence the germination of every kind of seeds (NASCIMENTO, 2004). In addition, the osmopriming process is more efficient when applied on seeds with low to medium vigor (NASCIMENTO; ARAGÃO, 2004; PEREIRA *et al.*, 2008).

Normally, araçá-boi seeds have as main characteristic a long germination period, which may reach up to eight months to get 100 % germination. Thus, it requires treatments to anticipate and uniform germination, and increase the speed of germination index for obtaining plantlets. In this context, the objective of this study was to evaluate the osmopriming efficiency promoted by sucrose ($C_{12}H_{22}O_{11}$) and potassium nitrate (KNO_3) on the germination of araçá-boi seeds in different immersion times.

MATERIAL AND METHODS

Seeds used in this study were obtained from araçá-boi ripe fruits collected in the city of Manaus, in November 7th to 9th in 2013. Seeds extraction was carried out manually, placed on a sieve and washed under running water until complete removal of mucilage. Afterwards, seeds were dried in natural environment conditions (average temperature = 25 °C) for two hours, and seeds with mass of 64 g were selected to arrange the experiments.

The seeds were placed in containers, immersed in distilled water, which was changed every three days until the experiment completion. A 4 × 3 factorial experiment with four osmopriming treatments and three conditioning times (6, 12 and 24 hours) as follow: : T1 - Sucrose solution (30 g L^{-1}); T2 - KNO_3 solution (4,5 g L^{-1} with osmotic potential of -1,1 MPa, and concentration based on the Van't Hoff equation (HILLEL, 1971); T3 - Sucrose solution + KNO_3; and T4 - distilled H_2O (Control). After the end of the experiment, seeds were taken from the containers and immersed in a NaClO solution at 2 % active chlorine, for 60 minutes, for sterilization. Then, seeds were washed vigorously in distilled water to remove the excess of NaClO and placed in plastic boxes (Gerbox®), previously lined with moistened filter paper with the osmopriming solutions (equivalent to 2.5 times the paper weight) according to each treatment. After that, the plastic boxes (Gerbox®) were kept in a B.O.D chamber (Biochemical Oxygen Demand) at 10 °C in the dark, and wrapped with plastic bag to prevent solution evaporation during the evaluation times.

After each conditioning period (6, 12 and 24 hours), seeds were washed in distilled water, and put back in plastic boxes (Gerbox®) previously lined with filter paper, adding distilled water to cover one third of the seeds in order to quantify the germination percentage. Then, seeds were incubated in B.O.D chamber at 25 °C for 70 days, and the germination percentage and speed of germination index were assessed every 7 days according to Maguire (1962):

$$IVG = \frac{N1}{D1} + \frac{N2}{D2} + \frac{Nn}{Dn}$$

Where N1, N2 and Nn = number of germinated seeds in the first, second, to the last count; and D1, D2 ... Dn =

number of days from sowing to the first, second and last count.

All data were first submitted to the normality and homogeneity tests. Then, the data were analyzed using analysis of variance (ANOVA) and the means from the treatments were compared by Tukey test ($P \leq 0.05$) using the software SISVAR (FERREIRA, 2011). For greater reliability, the experiment were repeated three times. The obtained data had similar results and a joint analysis was carried out.

RESULTS AND DISCUSSION

Significant effect was found for the factor osmopriming solution (Table 1). Based on the results obtained in the germination test, the osmopriming treatments using KNO_3 solution provided higher germination percentage (99 % of germinated seeds) when compared to other treatments until the 70th day, (Table 1).

Table 1 - Average values of germination percentage and speed of germination index of araçá-boi seeds after 70 days of osmopriming in different solutions

Solution	Germination percentage
KNO_3	99 a
$C_{12}H_{22}O_{11}$	37 b
$C_{12}H_{22}O_{11}$ + KNO_3	27 c
Destilled H_2O	7 d
	Speed of germination index
KNO_3	2.14 a
$C_{12}H_{22}O_{11}$	1.53 b
$C_{12}H_{22}O_{11}$ + KNO_3	1.51 b
Destilled H_2O	0.04 c

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

The speed of germination index of araçá-boi seeds during 70 days of evaluation was higher on seeds osmoprimed in KNO_3 solution when compared with the other solutions and with the control (Figure 1) As can be seen, osmopriming araçá-boi seeds with KNO_3 provided higher germination percentage and also favored higher speed of germination index (Table 1).

Normally, araçá-boi seeds have as main characteristic a long germination period, which may reach up to eight months to get 100 % germination. In the present study, it was found that seeds osmoprimed with KNO_3 solution initiated

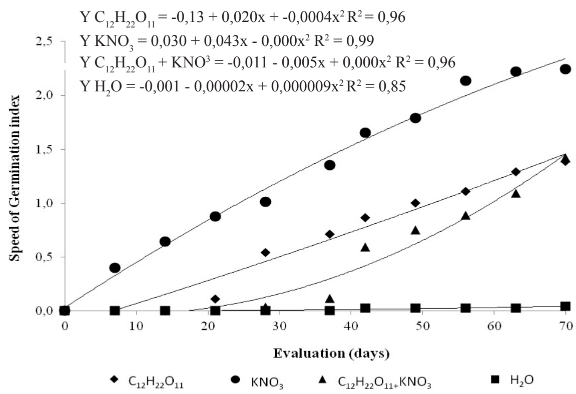

Figure 1 – Speed of germination index of araçá-boi seeds osmoprimed in different solutions during 70 days.

germination at 7 days after experiments establishment, followed by sucrose treatment (Figura 1). Seeds subjected to osmopriming only in water initiated germination between 42 and 49 days after settlement (Figure 1), and reaching a maximum of 7 % germination (Table 1).

Sert *et al*. (2009) found that leaching in water may be an effective method for removing the dormancy caused by inhibitors. Castro and Hilhorst (2004) also stated that water imbibition of seeds promotes faster and more uniform germination. Even that water contributed to leaching the inhibitor compounds, and in some instances, increase the germination percentage and rate, in this study, it was founf that the H_2O treatment presented the lowest results when compared with the reagent solutions. Depending on the species, speed of germination index may not be influenced by pre imbibition, which is a treatment that not necessarily accelerates seeds speed of germination index of every species (NASCIMENTO, 2004). This result is related to the characteristics of each species, to the lot and to other morphogenetic properties of seeds, even if osmopriming induced more significant effects in seeds of low and medium vigor, such as those used in this work. Seeds are of low vigor because seeds immersed and imbibed in distilled water did not present more than 7 % germination (Table 1 and Figure 1) after 70 days of evaluation.

Marcos Filho (2005), demonstrated that during osmopriming process using KNO_3, its molecules allow better aeration of the seed and penetration of salts ions inside the cells, which directly influences their metabolism. This may explain the treatments efficiency using KNO_3 in osmopriming of araça-boi seeds. Similar results were obtained by Rodrigues *et al*. (2012) studying the effect of KNO_3 in osmopriming lettuce seeds. The authors observed that the osmoprimed seeds showed increase in speed of germination index after treatment with KNO_3 solution.

Bell pepper seeds osmoprimed in KNO_3 solution also promoted increases on the speed of germination index, especially on seeds collected in fruits at early maturing stage (ALBUQUERQUE *et al*., 2009). However, in studies testing the influence of osmopriming using KNO_3 solution on the performance and seed germination on beet (COSTA; VILELA, 2006) and onion seeds (CASEIRO; MARCOS FILHO, 2015), the results shown a different response, promoting a reduction. Increase on germination efficiency promoted by osmopriming in melon and carrot seeds of low vigor was observed by Nascimento and Aragão (2004) and Pereira *et al*. (2008), respectively. On the other hand, lettuce and coffee seeds with high viability, when subjected to osmopriming, showed no increase in the germination efficiency (FESSEL *et al*., 2001; PERTEL *et al*., 2001). This demonstrates that osmopriming process efficiency can vary according to the species tested.

CONCLUSIONS

Osmopriming of araçá-boi seeds in KNO_3 solution is efficient to promote germination and to increase speed of germination index;

Araçá-boi seeds osmoprimed in KNO_3 presented 99 % germination after 70 days of evaluation;

Osmopriming period does not influence the germination of araçá-boi seeds.

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