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Use of phytoregulators in overcoming macaw palm seed dormancy

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ABSTRACT. The macaw palm is a tropical palm with significant potential for biofuel production; however, seed dormancy is a major factor limiting its agro-industrial use. The present study evaluated the effects of phytoregulators in overcoming macaw palm seed dormancy. We evaluated the effects of immersion in solutions of gibberellic acid (GA_3) (0, 2000 and 5000 mg L^{-1}) during two exposure times (24 and 48 hours), associated with the removal or maintenance of the opercular tegument, as well as the effects of the associations between GA_3 (2000 mg L^{-1}), indole-3-butyric acid (IBA) and benzylaminopurine (BAP) and the effects of repeated applications (one, two or five) of combinations of these phytoregulators. The seeds were sown in vermiculite and incubated in a humid growth chamber at 95 ± 5% relative humidity and 30°C for 18 weeks in all experiments. GA_3 application and removal of the opercular tegument had positive effects on germination, but no significant differences were observed in immersion times for this phytoregulator. The application of IBA and BAP did not influence germination. The application of GA_3 on five separate occasions gave the best results, with 41% germination at the end of the experiment.

Keywords: *Acrocomia aculeata*, germination, GA_3, IBA, BAP, Arecaceae.

Uso de fitorreguladores na superação da dormência em sementes de macaúba

RESUMO. A macaúba é uma palmeira tropical com grande potencial para produção de biocombustíveis. Entretanto a dormência nas sementes limita o aproveitamento agroindustrial da espécie. O presente estudo avaliou o efeito da aplicação de fitorreguladores na superação da dormência em sementes de macaúba. Avaliou-se o efeito de soluções de ácido giberélico (GA_3) (0, 2000 e 5000 mg L^{-1}), em dois tempos de imersão das sementes (24 e 48 horas), associadas à manutenção ou retirada do tegumento opercular, bem como o efeito da associação entre GA_3 (2000 mg L^{-1}), ácido indol-3-butírico (AIB) e benzilaminopurina (BAP) e o efeito da quantidade de aplicações (uma, duas ou cinco) das combinações dos fitorreguladores. Em ambos os experimentos, as sementes foram plantadas em vermiculita e a germinação foi conduzida em câmara úmida com 95 ± 5 % de UR, a 30°C, durante 18 semanas. Observou-se efeito positivo da aplicação de GA_3 e da retirada do tegumento opercular, não havendo diferenças significativas entre os tempos de imersão. A imersão em AIB e BAP, não influenciou a germinação. A aplicação de GA_3 por cinco vezes separadas proporcionou os maiores percentuais de germinação, atingindo 41% ao final do experimento.

Palavras-chave: *Acrocomia aculeata*, germinação, GA_3, AIB, BAP, Arecaceae.

Introduction

Acrocomia aculeata (Jacq.) Lodd. ex. Mart. (macaw palm) is an oleaginous palm tree that is native to the tropical Americas and has a wide geographical distribution; it is especially common in south-eastern Brazil (LORENZI et al., 2004; MOTTA et al., 2002). This species has significant potential for producing industrial quantities of biofuel because of its high productivity and tolerance to dry environments (CLEMENT et al., 2005; MOURA et al., 2009). Seed dormancy, however, restricts large-scale commercial plantings and its extensive agro-industrial use (RIBEIRO et al., 2011).

Dormancy is the intrinsic blockage of the germination of viable seeds under conditions in which non-dormant seeds germinate (FINCH-SAVAGE; LEUBNER-METZER, 2006; LINKIES et al., 2010). Delayed seed germination in palms has been associated with morphological dormancy due to embryo immaturity (BASKIN; BASKIN, 1998; OROZCO-SEGOVIA et al., 2003) as well as physiological dormancy. Gibberellic acids (GAs) promote the germination of macaw palm seeds, indicating the existence of physiological dormancy (RIBEIRO et al., 2011). Additionally, embryos of this species that are cultivated *in vitro* germinate rapidly, indicating that their degree of maturation is not a limiting factor (RIBEIRO et al., 2012) and that their physiological dormancy is of the not deep type (BASKIN; BASKIN, 2004).

Numerous studies have demonstrated the efficiency of gibberellins in stimulating germination and overcoming physiological dormancy in palm seeds (CHIN et al., 1988; DEWIR et al., 2011; HERRERA et al., 1998; NAGAO et al., 1980; PÉREZ et al., 2008; ROBERTO; HABERMANN, 2010; YANG et al., 2007), but other factors can also influence the effectiveness of this hormone treatment. The quantity of gibberellin that is necessary to promote germination depends on the degree of restriction of embryo elongation imposed by the endosperm and tegument (DEBEAUJON; KOORNNEEF, 2000); the removal of either the operculum or opercular tegument is efficient in promoting germination in numerous palm species (CARPENTER et al., 1993; HUSSEY, 1958; PÉREZ et al., 2008). On the other hand, Nagao et al. (1980) demonstrated that the external tissues in *Archontophoenix alexandrae* (F. Muell.) H. Wendl. Drude and *Ptychosperma macarthurii* H. Wendl. restricted GA_3 penetration and that the removal of these tissues significantly increased the effects of this phytoregulator.

Ribeiro et al. (2011) reported that immersing macaw palm seeds in 2000 mg L^{-1} of GA_3 for 24 hours in association with removal of the opercular tegument resulted in germination indices of 52% with the re-application of the phytoregulator. However, the effects of higher doses of this phytoregulator and the effects of longer periods of seed immersion were not evaluated; such treatments could increase germination and contribute to the establishment of protocols for commercial propagation programs.

There are many examples of the effects of plant hormones on germination and/or their interaction with gibberellins (BEWLEY; BLACK, 1994; KUCERA et al., 2005), and several studies have evaluated the effects of cytokinins on palm seed germination (VILLALOBOS et al., 1992; ROBERTO; HABERMANN, 2010). Ledo et al. (2001) demonstrated that the association of 1-naphthaleneacetic acid (NAA) and BAP had positive effects on the *in vitro* germination of zygotic embryos of *Euterpe oleraceae* Mart. Studies evaluating the interactions between GA_3 and other phytoregulators may enhance our knowledge of *A. aculeata* seed germination.

The objective of this study was to evaluate the effect of the application of phytoregulators, the removal of the opercular tegument and the association between these treatments on macaw palm seed germination.

Material and methods

Characterisation of the seed lots

Fruits of *A. aculeata* were harvested soon after natural abscission from the wild population in the municipality of Montes Claros, Minas Gerais State, Brazil (16°42'34"S; 43°52'48"W) and were transported to the Micropropagation Laboratory of State University of Montes Claros, where the experiments were conducted. Two different lots of seeds that were collected at different times were used in the experiments; these lots were identified as lots 1 and 2. The lots were stored in the shade for five and four months, respectively. To obtain the seeds, the fruits were broken with the aid of a bench-top vice; damaged seeds were discarded. The seeds were surface-sterilised in a 6% chlorine solution for 10 min., followed by three rinses in running water. The water contents of the seeds in each lot were determined by drying them for 24 hours at 105°C (BRASIL, 2009).

Effects of the removal of the opercular tegument, GA_3 dose and immersion time

The seeds were maintained in polystyrene trays containing vermiculite for seven days in a humid growth chamber at 95 ± 5% relative humidity at 30°C. The opercular tegument was removed from half of the tested seeds using a razor blade under a stereomicroscope (RIBEIRO et al., 2011). The seeds were subsequently immersed for 24 or 48 hours in aqueous solutions containing 0, 2000 or 5000 mg L^{-1} of GA_3 (PROGIBB®) and then returned to the humid growth chamber. The seeds were aerated using an air pump during immersion. All possible combinations of the presence or absence of the opercular tegument, GA_3 concentrations, and immersion times were tested, with five repetitions of 20 seeds per treatment. The seeds were evaluated on a weekly basis for 18 weeks for germination (or deterioration) to calculate the germination percentage (%G) and germination velocity index (GVI). Germination was defined as the visible protrusion of the cotyledon petiole (BEWLEY; BLACK, 1994); the presence of necrosis or fungal mycelia was indicative of deterioration.

Effects of interactions between phytoregulators and the number of applications

The opercular teguments were removed from the seeds, and the effects of seed immersion in 2000 mg L^{-1} GA_3 (PROGIBB®), or its absence along with four combinations of IBA and BAP (Sigma®) were evaluated (Table 1). Seed immersion was performed in plastic cups containing 100 mL of aqueous solutions of phytoregulator combinations, with alternating applications of GA_3 and IBA/BAP combinations applied each week. Each combination of GA_3, IBA, and BAP was applied one, two or five times. These experiments were conducted under the same conditions as the previous experiment, testing all possible combinations of GA_3, IBA and BAP concentrations with five repetitions of 20 seeds per treatment. Seed germination was evaluated as described earlier, and seeds that did not germinate until the end of the evaluation period were treated with 0.5% tetrazolium to determine their viability, following the protocol of Ribeiro et al. (2010).

The data from both experiments were submitted to analyses of variance, and the Tukey test was used to compare their averages (SAS INSTITUTE, 1990). The values corresponding to per cent germination and deterioration were arc sine converted (x $100^{-1})^{0.5}$ for comparison.

Table 1. Combinations of IBA and BAP applied to *A. aculeata* seeds in association with GA_3.

Combination	IBA (mg L^{-1})	BAP (mg L^{-1})
C1	0	0
C2	500	0
C3	500	100
C4	500	500

Results and discussion

Effects of the removal of the opercular tegument, GA_3 dose and immersion time

The seed lot used in these experiments (lot 1) had a water content of 6.6% before pre-imbibition. After the treatment, the water content of the seed lot reached 22%. The start of germination was observed in the second week after the GA_3 immersion treatments. The duration of immersion in GA_3 did not influence the germination percentage. Significant effects were observed resulting from the removal of the opercular tegument, different doses of GA_3, and in the interactions among these variables (Figure 1A). The removal of the opercular tegument increased germination independent of the GA_3 concentration; thus, this phytoregulator was effective only in seeds with intact opercular teguments. The %G of seeds immersed in 2000 and 5000 mg L^{-1} were not significantly different, but they were both higher than the control.

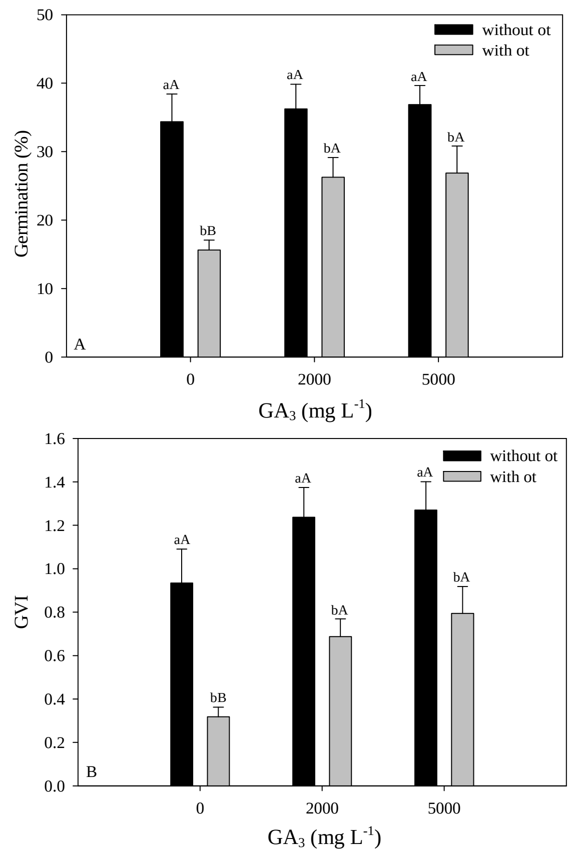

Figure 1. (A) Germination percentages (%G) and (B) germination velocity index (GVI) of *A. aculeata* seeds with or without the opercular tegument (OT) after immersion in solutions with different concentrations of GA_3. Identical lower case letters indicate the absence of significant differences between seeds with or without OT in each concentration of GA_3. Identical upper case letters indicate the absence of any significant differences between GA_3 concentrations in seeds with or without OT by the Tukey test at a 5% level of probability. Bars represent the standard error of mean.

Positive effects were observed with respect to the GVI, resulting from the removal of the opercular tegument, immersion in GA_3, and interactions between the variables (Figure 1B). The GVI of seeds immersed in 2000 and 5000 mg L^{-1} were not significantly different but were higher than those of the control. Immersion in GA_3 for 48 hours resulted in the highest GVI, but there were no significant interactions between the immersion duration and the other variables. Seed deterioration was approximately 18% and was significantly influenced by immersion in GA_3 ($p = 0.0002$).

The removal of the opercular tegument results in increases in germination percentages in other palm species (CARPENTER et al., 1993; HUSSEY, 1958; PÉREZ et al., 2008). The efficacy of this treatment is most likely related to the decreased resistance to embryonic elongation that is normally imposed by the opercular tegument.

Gibberellins are known to aid in overcoming seed dormancy by stimulating embryo development and by the production of hydrolases that weaken seed structures adjacent to the embryo (HOOLEY, 1994; YAMAGUCHI; KAMIYA, 2002). Increased macaw palm seed germination after exposure to GA_3 was reported by Ribeiro et al. (2011), and other studies have shown similar effects in other palm trees (CHIN et al., 1988; DEWIR et al., 2011; HERRERA et al., 1998; NAGAO et al., 1980; PÉREZ et al., 2008; ROBERTO; HABERMANN, 2010; YANG et al., 2007).

Ribeiro et al. (2011) reported that the removal of the opercular tegument associated with a single application of 2000 mg L^{-1} GA_3 resulted in increased germination, as was observed in the present study. These authors also noted that repeated exposure to GA_3 for four weeks after sowing resulted in even higher germination percentages, and they suggested that the positive effects of the reapplication of this hormone were due to an increasing sensitivity of the seeds to GA_3 over time; alternatively, 24 hours of exposure was insufficient to promote seed germination. Our results indicate that the effects of immersion in GA_3 for 24 hours on seed germination were not significantly different from the results of 48 hours of immersion. Likewise, immersion in 2000 or 5000 mg L^{-1} GA_3 did not result in significant differences in germination. As such, the positive effects of the re-application of GA_3 does not indicate that either the 2000 mg L^{-1} concentration or immersion for 24 hours were insufficient, in agreement with the hypothesis of increased sensitivity to GA_3 over time.

The levels of seed deterioration observed in experiments involving exogenous GA_3 (17.8%) were very close to those reported by Ribeiro et al. (2011), who considered this problem a roadblock to the commercial propagation of macaw palm.

Effects of interactions between phytoregulators and the number of applications

The seeds used in this experimental procedure (lot 2) had water contents of 8% at the beginning of the experiments, but by two weeks after the tests, their water contents had reached 20%. As in the previous experiment, germination starts in the second week after exposure to GA_3. Immersion in IBA and BAP had no effect on the germination percentages, although immersion in GA_3 resulted in a significantly higher germination percentage than that of the controls, reaching 41% after the fifth application. When applications of the other phytoregulators (AIB and BAP) were increased in the absence of GA_3, there was no increase in the germination percentage above that of the control. On the other hand, when GA_3 was applied five times, a significant increase in %G was observed (Figure 2A).

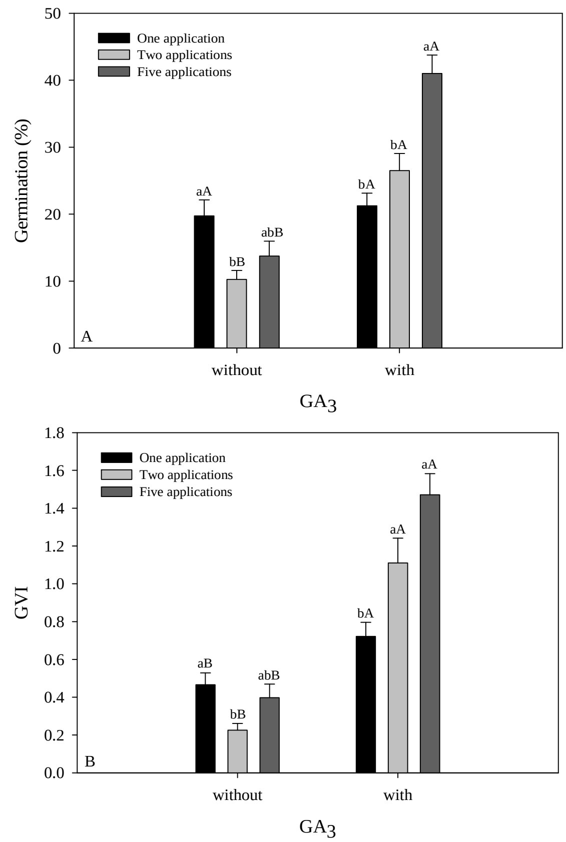

Figure 2. (A) Germination percentages (%G) and (B) germination velocity index (GVI) of *A. aculeata* seeds as a function of GA_3 and the numbers of times the phytoregulator was applied. Identical lower case letters indicate the absence of any significant differences between the numbers of applications in each treatment (with or without GA_3). Identical upper case letters indicate the absence of any significant differences between treatments (with or without GA_3) with identical numbers of applications, using the Tukey test at a 5% level of probability. Bars represent the standard error of mean.

Seeds that were immersed in GA_3 demonstrated greater GVI independent of the quantities applied (Figure 2B). Immersion in IBA and BAP did not significantly influence the GVI. Seed deterioration was approximately 35% and was influenced by GA_3 treatment ($p < 0.001$); tetrazolium tests demonstrated that 92.5% of the seed embryos that had not deteriorated remained viable.

Increasing numbers of applications of GA_3 resulted in higher germination percentages, as was observed by Ribeiro et al. (2011), and may be related to seed heterogeneity. Germinability in palms varies considerably as a function of the year that the seeds were collected and among individual seeds (OROZCO-SEGOVIA et al., 2003); seeds from the same lot can demonstrate different degrees of dormancy (BASKIN; BASKIN, 1998). This innate irregularity in overcoming dormancy makes germination studies difficult because the stimuli required to initiate germination can vary greatly (BEWLEY, 1997). As such, the positive effects of the re-application of GA_3 may be associated with the asynchronous manner in which dormancy is overcome in each seed lot. With respect to the seeds that demonstrate physiological dormancy, the quantity of GA_3 necessary to promote germination will be dependent on the degree of restriction imposed on embryo elongation by the structural characteristics of the endosperm and tegument, which may vary in function for each individual and environmental condition as well as over time (DEBEAUJON; KOORNNEEF, 2000; FINCH-SAVAGE; LEUBNER-METZGER, 2006).

In addition to the differences in the degree of dormancy among seeds of the same lot, the positive effects of the reapplication of GA_3 may be related to their endogenous levels of abscisic acid (ABA). ABA is a positive regulator of dormancy induction and a negative regulator of germination (KUCERA et al., 2005; NAMBARA et al., 2010). The seeds in this study were submitted to warm stratification in a humid growth chamber, which could have provoked decreases in ABA levels over time, resulting in variations in the responses of those seeds to GA_3. Chien et al. (1998) reported decreases in ABA concentrations in *Taxus mairei* (Taxodiaceae) seeds during warm stratification, and the same process could have occurred in the *A. aculeata* seeds tested in the present study. This hypothesis is corroborated by experiments with various other species that have demonstrated that incubating seeds at high temperatures in humid environments overcomes physiological dormancy and increases sensitivity to external applications of GA_3 (BASKIN; BASKIN, 1998; FINCH-SAVAGE; LEUBNER-METZGER, 2006). Pérez et al. (2008) reported that removal of the operculum followed by incubation at high temperatures (25-35ºC) resulted in high germination percentages in *Pritchardia remota* palm seeds. Additionally, high-temperature treatments promoted decreases in ABA levels in the seeds of *Elaeis guineensis* palms (JIMENEZ et al., 2008), making it an effective treatment for overcoming dormancy in that species (HUSSEY, 1958; REES, 1962). Another factor that should be considered is the diffusion velocity of GA_3 to the seed interior. Nagao et al. (1980) observed that gibberellin effectiveness was limited by its ability to penetrate the seed tegument. Slow diffusion would necessarily require longer exposure times or repeated hormone applications at later times, allowing the seeds to absorb sufficient quantities of GA_3 to promote germination. As such, the efficiency of the re-application of GA_3 might be a reflection of its slow absorption by the seeds.

The effects of immersion in auxin solutions (such as IBA) on seed germination have not been well studied, and little is known about the role of auxins in seed germination at the molecular level (KUCERA et al., 2005). However, Jimenez et al. (2008) observed that the levels of indole acetic acid (IAA) in *Elaeis guineensis* seeds increased considerably during imbibition, suggesting that this hormone plays an important role in germination in that species.

The endosperm has been described as a source of cytokinins that are necessary to promote embryonic cell division (KUCERA et al., 2005). A number of studies have provided evidence that cytokinins, like gibberellins, might aid in overcoming ABA-imposed inhibition (FOUNTAIN; BEWLEY, 1976), so that in some species, the simple use of cytokinins is sufficient to overcome seed dormancy (KUCERA et al., 2005). On the other hand, immersion in BAP did not result in increased germination in *Euterpe edulis* (ROBERTO; HABERMANN, 2010) and *Bactris gasipaes* (VILLALOBOS et al., 1992) palms, as was observed in *A. aculeata* in the present study.

The present experiments resulted in even higher deterioration levels (34.8%), which might be related to the effects of the weekly immersions to which the seeds were submitted. These results highlight the importance of additional studies concerning microbial control in perfecting the propagation technologies for *A. aculeata*.

Conclusion

The immersion of *A. aculeata* seeds in 2000 or 5000 mg L^{-1} GA_3 and the removal of the opercular tegument result in increased germination.

The positive effects of immersion in GA_3 tended to increase with increasing numbers of applications.

Exposure to IBA and BAP was ineffective in promoting the germination of *A. aculeata* seeds.

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