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Effects of some mechanical and chemical treatments on seed germination of *Sabal palmetto* and *Thrinax morrisii* palms

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Abstract

Sabal palmetto and *Thrinax morrisii*, Arecaceae, are important ornamental palms of socioeconomic importance. Experiments using physical, mechanical and chemical pre-sowing treatments were conducted to determine the germination response of these two palm species. Among various treatments, soaking of *Sabal* seeds in 500 ppm gibberellic acid (GA_3) for 24 h resulted in a highest final germination percentage (FGP) of 95% in day 14 of culture and number of days lapsed to reach 50% of FGP (GT_{50}) of 6.8 days. Non-treated *Sabal* seeds exhibited FGP of 75% in day 16and GT_{50} of 7.39 days. Soaking of *Thrinax* seeds in H_2SO_4 for 30 min resulted in a highest FGP of 90% in day 14 of culture and GT_{50} of 5.19 days. Non-treated *Thrinax* seeds exhibited FGP of 70% in day 16 and GT_{50} of 8.07 days. The results indicate that *Thrinax* seeds exhibit exogenous dormancy which is entirely imposed by the hard seed coat, whereas the *Sabal* seeds exhibit both exogenous and physiological dormancy.

Keywords: Arecaceae, Dormancy, Germination, Palms, Scarification, Seed coat
Abbreviations: CGRI – Corrected germination rate index; FGP – Final germination percentage; GA_3 – Gibberellic acid; GRI – Germination rate index; GT_{50} – Number of days lapsed to reach 50% of FGP

Introduction

The palms are a globally important family of socioeconomic plants. The Arecaceae family includes palm trees of economical importance both as a source of agricultural produce, as well as ornamental components in landscaping projects (Henderson et al., 1995). *Sabal palmetto*, the cabbage palm, is native to the Western Hemisphere. It attains a height at maturity of 12 to 27 m and has been planted widely as an ornamental tree (Sargent, 1965). It has few commercial uses but is used extensively by rural residents for a variety of purposes; the trunk for timber, the bud for food, and the leaves for craft weaving. *Thrinax morrisii*, the key thatch palm, is an evergreen, single-stemmed shrub or small tree 1 to 6 m in height. It is native to southern Florida and the West Indies. *Thrinax* palm helps protect the soil, contributes to the aesthetics of natural stands, and furnishes food and cover for wildlife. The species is grown and sold as an ornamental (Gilman and Watson, 1994). Leaves are used to make brooms, thatch, and mats (called petate) (Osvaldo, 2002), although now for ornamentation rather than necessity. The fruit pulp contains 4,083 µg/g of oxalate - capable of causing a burning sensation on the skin of sensitive people (Broschat and Meerew, 2000). Palms are unique among woody ornamental plants because, with relatively few exceptions, palm species can only be propagated from seeds. Palm seeds are generally considered to be short lived and often lose its viability after 2 – 3 months of storage (Deleon, 1958; Broschat and Donselman, 1987). It has been estimated that over 25% of all palm species require over 100 days to germinate and has less than 20% total germination (Tomlinson, 1990). Due to the often slow and uneven germination of palm seeds, there has been a great deal of interest in any preplant treatments that might speed germination or result in more even rates of germination (Moussa et al., 1998; Pinheiro, 2001; Potvin et al., 2003; Gomes et al., 2006; Yang et al., 2007; Klinger and Rejmánek, 2010). However, little investigations have been accomplished on the mechanism of seed dormancy conditions in *Sabal* and *Thrinax* palms. As a result, these palms are notorious in the nursery trade for slow and uneven seed germination. This information is important for the commercialization of *S. palmetto* and *T. morrisii* as ornamental species. Considering that in practice the propagation of palm plants is accomplished mainly by seeds, the objectives of this study were to investigate this mechanism, and to find methods to break dormancy for achieving rapid, uniform and high germination.

Materials and methods

Seed materials

The fruits of *Sabal palmetto* and *Thrinax morrisii* were obtained from the Antoniadis garden, Alexandria- Egypt in June 2009. *Sabal* fruits were dark-brown to black whereas *Thrinax* fruits were white and fleshy. The fruits were round and approximately 14 mm and 10 mm in diameter for *Sabal* and *Thrinax*, respectively. The fresh weight of 100 Sabal seeds was 50.316 g and the moisture content was 88.9%

Table 1. Effect of pre-sowing seed treatments on final germination percentage (FGP), germination rate index (GRI), corrected germination rate index (CGRI) and number of days lapsed to reach 50% of final germination percentage (GT_{50}) in *Sabal palmetto* after 30 days in culture

Treatments	FGP	GRI	CGRI	GT_{50}
			Intact seeds	
Control	75 c[Z]	62.15 e	80.29 fgh	7.39 bc
Water soak (24 h)	95 a	68.02 de	71.89 h	9.53 a
Mechanical scarification	75 c	94.56 bc	125.47 bc	4.32 de
H_2SO_4 (5 min)	85 b	109.46 ab	132.49 b	4.44 de
H_2SO_4 (15 min)	75 c	115.98 a	149.52 a	3.11 e
H_2SO_4 (30 min)	60 d	61.18 de	139.28 bc	3.67 e
			Scarified seeds	
1% KNO_3	90 a	85.69 cd	96.64 def	6.65 bc
2% KNO_3	65 d	63.81 e	95.66 ef	6.77 bc
4% KNO_3	60 d	66.39 e	113.42 cd	5.74 cd
GA_3 100 ppm	60 d	58.01 e	98.71 de	5.82 cd
GA_3 250 ppm	80 b	61.21 e	75.71 gh	8.35 ab
GA_3 500 ppm	95 a	84.95 cd	89.88 efg	6.80 bc

[Z]Mean values followed by different letters within each column are significantly different based on Duncan's Multiple Range test at 5% level

while the fresh weight of 100 *Thrinax* seeds was 116.885 g and the moisture content was 87.4. The moisture content was estimated after drying the seeds at 100 °C for 16 h (Yang et al., 2007). The pulp of the fruit was removed by rubbing them against a sieve. Next, the pyrenes (which consisted of the endocarp and seed) were washed in running water and dried in shade for a day. All seeds were surface sterilized in 70% ethanol for 30 sec. and later in 0.1% mercuric chloride with a few drops of wetting agent Tween-20 (polyoxy-ethylene-sorbitan monolaurate) for 20 min. then washed with sterile distilled water for five times to prevent contamination.

Pre-sowing seed treatments

Seeds were subjected to different mechanical, physical and chemical treatments. Mechanical scarification was achieved by vigorously rubbing the seeds between two sheets of fine-grained sand paper to remove the testa without injuring the embryo (Pérez-García and González-Benito, 2006). Physical scarification was carried out by soaking intact seeds in distilled water for 24 h at ambient temperature. Chemical scarification was accomplished using three different techniques. Firstly, samples of intact seeds were soaked separately in concentrated sulphuric acid (97% H_2SO_4) for 5, 15 and 30 min. Secondly, samples of mechanically scarified seeds were soaked separately in potassium nitrate (KNO_3) at 0, 1, 2 and 4% for 24 h. Finally, samples of mechanically scarified seeds were soaked separately in gibberellic acid (GA_3) at 0, 100, 250 and 500 ppm for 24 h. All seeds in the H_2SO_4, KNO_3 and GA_3 treatments were thoroughly washed in sterile distilled water before culturing. Intact seeds without pre-sowing treatments were considered as the control.

Experimental design and culture conditions

All experiments were conducted in a completely randomized design. There were 12 treatments consisting of 3 replications and each replication represented by 10 seeds. Seeds were placed in sterile glass Petri dishes (9 cm) containing 20 ml of 1% agar. The dishes were sealed with clear plastic to prevent evaporation during the experiment. All cultures were incubated for 30 days at 16 h photoperiod provided by a fluorescent light at 40 μmol $m^{-2}s^{-1}$ and a constant temperature of 25 °C.

Data collection and statistical analysis

Germinated seeds were counted every 48 h and then discarded. A seed was considered germinated when the tip of the radical had grown free of the seed coat (Wiese and Binning, 1987; Auld et al., 1988). The following germination parameters were recorded: (a) Germination percentage (GP) = (number of germinated seeds/number of tested seeds) × 100.

(b) Germination rate index (GRI) = $[(G_1/1) + (G_2/2) + (G_x/X)]$

where, G = germination on each alternate day after placement 1, 2, x = corresponding day of germination (Esechie, 1994)

(c) Corrected germination rate index (CGRI) = (GRI/FGP) × 100

where, FGP = final germination percentage

(d) GT_{50} = number of days lapsed to reach 50% of FGP (Hsu et al., 1985)

Experiments were set up in a completely randomized design. The mean and one-way ANOVA were calculated using SPSS (version 10) software. The mean separations were carried out using Duncan's multiple range tests (Duncan, 1955) and significance was determined at $p \leq 0.05$.

Results and discussion

Effect of physical, mechanical and H_2SO_4 scarification of the intact seeds on changes in germination percentage of Sabal palmetto and Thrinax morrisii

The effects of various pre-sowing seed treatments on the time-course changes in germination percentage of *Sabal* and *Thrinax* are shown in Fig. 1 and 2, respectively. For the two species studied, the watersoaking (24 h) and H_2SO_4 treatments increased the germination percentage of *Sabal* and *Thrinax* respectively. The water soaking (24 h) treatment of *Sabal* and mechanical scarification of *Thrinax* were better than the control and reached 95% and 85%, respectively (Fig. 1a and 2a). This is in accordance with studies by Moussa et al. (1998) on freshly-collected doum palm seeds.

Table 2. Effect of pre-sowing seed treatments on final germination percentage (FGP), germination rate index (GRI), corrected germination rate index (CGRI) and number of days lapsed to reach 50% of final germination percentage (GT_{50}) in *Thrinax morrisii* after 30 days in culture

Treatments	FGP	GRI	CGRI	GT_{50}
Intact seeds				
Control	70 c[Z]	56.99 cd	81.72 cde	8.07 bc
Water soak (24 h)	45 g	24.85 e	54.67 f	12.45 a
Mechanical scarification	85 b	73.31 b	85.93 bcd	7.44 bc
H_2SO_4 (5 min)	65 d	60.88 bcd	98.69 ab	6.76 cd
H_2SO_4 (15 min)	75 c	68.31 bc	93.27 abc	7.31 bc
H_2SO_4 (30 min)	90 a	95.90 a	106.48 a	5.19 d
Scarified seeds				
1% KNO_3	40 g	31.57 e	78.93 cde	8.50 bc
2% KNO_3	65 d	50.35 d	78.56 de	8.07 bc
4% KNO_3	80 b	57.47 cd	71.71 de	8.97 b
GA_3 100 ppm	65 d	48.34 d	74.89 de	8.64 b
GA_3 250 ppm	60 d	46.67 d	82.51 cde	8.17 bc
GA_3 500 ppm	50 e	33.60 e	68.21 e	11.00 a

[Z]Mean values followed by different letters within each column are significantly different based on Duncan's Multiple Range test at 5% level

Mechanical scarification and water soaking had germination rates higher than the equivalent unsoaked seeds. They also indicated that the impermeability of the pericarp to water and possibly oxygen was a major constraint on germination. The responses of *Sabal* to watersoaking and *Thrinax* to mechanical scarification that observed are similar to those observed by Todaria and Negi (1992), Cavanagh (1987), Teketay (1996), Prins and Maghembe (1994), Maithani et al., (1991) and Demel, (1998) for a number of different *Acacia* species, a common hard-seeded genus. According to Sy et al. (2001), immersion in H_2SO_4 could either be insufficient or lead to damage, depending on integumental resistance. Immersion of *Sabal* seeds in H_2SO_4 for 5 min and *Thrinax* seeds for 30 min resulted in germination percentages that were considerably higher than the control during the entire culture period and reached 85% and 90%, respectively (Fig. 1b and 2b). Similar results were obtained in studies carried out on doum palm seeds (Moussa et al., 1998) and in four African *Acacia* species (Masamba, 1994) showed that H_2SO_4 pretreatment alone resulted in 97% germination. The mechanical scarification and H_2SO_4 pretreatments are known to result in rapid, uniform and high germination for many species (Cavanagh, 1987). The mechanical scarification and water soaking effectively broke the physical dormancy of freshly collected doum palm seeds (Moussa et al., 1998). In a similar manner, our results showed that H_2SO_4 appears to have had a corrosive physical action on *Sabal* and *Thrinax* seeds. As the *Sabal* seed coat is thinner than *Thrinax*, possibly a shorter soaking duration of 5 min was effective for *Sabal*. Prolonged soaking of the seeds in H_2SO_4 possibly imposes injury to the embryo and simply burnt the seeds. Whereas longer soaking duration of 30 min in H_2SO_4 was more effective for *Thrinax* seed germination

Effect of KNO_3 and GA_3 treatments of mechanical scarified seeds on changes in germination percentage of Sabal palmetto and Thrinax morrisii

There was a significant increase in germination percentage with decreasing KNO_3 and increasing GA_3 concentrations for *Sabal*, whereas in *Thrinax* there was opposite trend of the same treatments. The nitrates and gibberellins have been widely used to overcome seed dormancy (Nadjafi et al., 2006; Çirak et al., 2007). Its application in the present study indicated that seed dormancy in the two plants is due to different reasons. Ortega-Baes and Rojas-Are´chiga (2007) suggested that lack of a response to GA_3 of *Trichocereus terscheckii* seeds do not have any physiological dormancy. Among different KNO_3 and GA_3 treatments of *Sabal*, the best germination percentages were achieved in 1% and 500 ppm respectively in which maximum germination of 90% and 95% were attained respectively (Fig. 1c and 2d). It is well-known that GA_3 increases and synchronizes seed germination of many plant species (Bewley and Black, 1994; Choudhary et al., 1996). It is thought that GA_3 somehow increases the physiological activity of the embryo (Farmer, 1997). Nagao et al. (1980) suggested a combination of water soaking (24 h) and GA_3 treatment (1000 ppm) for *Archontophoenix alexandrae* and *Ptychosperma macarthurii* seeds before sowing. In addition, (Moussa et al., 1998) found that 500 ppm GA_3 resulted in 57% germination of doum palm seeds. Also nitrogen compounds can break seed dormancy by decreasing C6/Cl ratio of CO and changing metabolic pathway, so they are usually used as germination accelerator (Bewley and Black, 1994). *Thrinax* seeds had different trend when compared with *Sabal* seeds of KNO_3 and GA_3 treatments. KNO_3 at 4% significantly hastened germination compared to the control, but low concentrations did not. Also, GA_3 was not required to break seed dormancy of *Thrinax*. Similar results were obtained by Yang et al. (2007) on *Areca triandra* Roxb.

Effect of pre-sowing treatments on FGP, GRI, CGRI and GT_{50} percentages of Sabal palmetto and Thrinax morrisii

The effect of various pre-sowing treatments on FGP, GRI, CGRI and GT_{50} percentages in *Sabal palmetto and Thrinax morrisii* is presented in Table 1 and 2. The highest FGP of *Sabal* intact seeds were 95% when soaked in water for 24 h while the highest FGP of scarified seeds were 90% and 95% when soaked in 1% KNO_3 and 500 ppm GA_3, respectively compare to the control at 75%. This response to the chemical and physical scarification provides evidence that the seed coat is the main inhibitor of germination (Shaik et al., 2008). The FGP significantly decreased with increasing the exposure to H_2SO_4 from 5 min to 30 min as well as KNO_3 concentra-

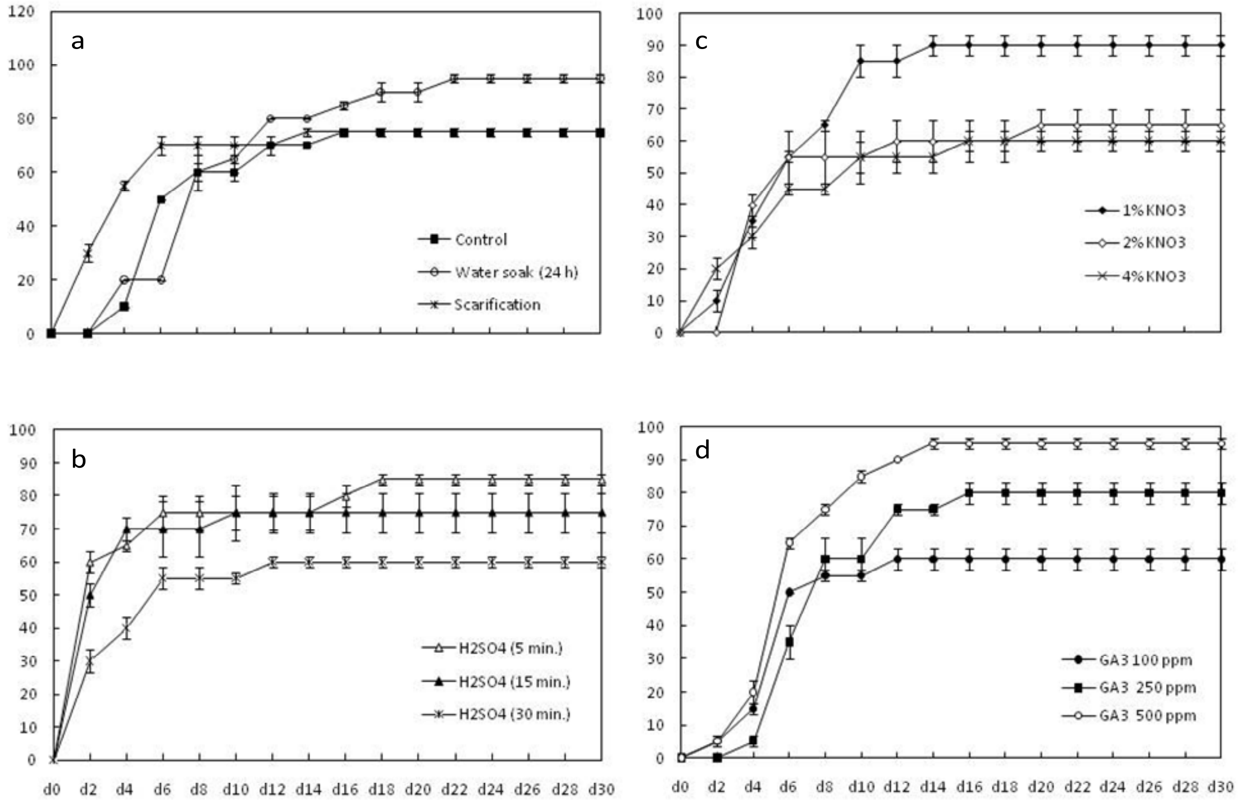

Fig 1. Time-course changes in germination percentage of *Sabal palmetto* seeds as affected by different pre-sowing treatments over 30 days of culture. a) scarification, water soak and the control; b) sulphuric acid; c) potassium nitrate d) gibberellic acid

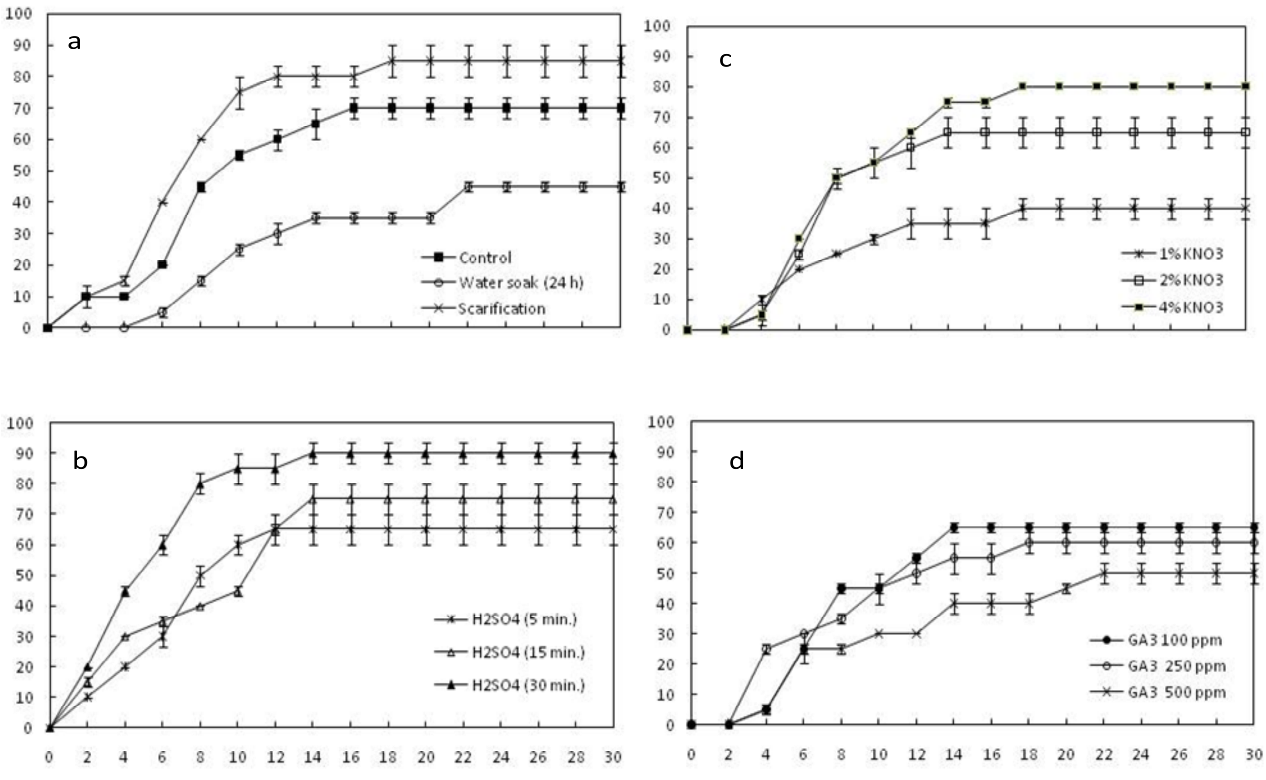

Fig 2. Time-course changes in germination percentage of *Thrinax morrisii* seeds as affected by different pre-sowing treatments over 30 days of culture. . a) scarification, water soak and the control; b) sulphuric acid; c) potassium nitrate d) gibberellic acid

tions from 1% to 4%. In contrast, FGP significantly increased with increasing GA_3 concentrations. Moussa et al. (1998) found on doum palm that both the seeds with their pericarp removed and the nuts bare responded similarly to GA_3 pretreatment which was higher than that obtained with H_2SO_4 treatment. The FGP of mechanical scarification treatment was not significantly different from FGP of the control. The combination of chemical treatments with mechanical scarification improved germination of *Sabal* seeds than mechanical scarification alone. This indicates that the physical barrier to oxygen and water movement imposed by the pericarp was not the only factor limiting germination in *Sabal* seeds. Germination also appeared to be improved by stimulating physiological activity of the embryo with either phytohormones or water soaking prior to sowing (Moussa et al., 1998). The FGP of *Thrinax* seeds significantly increased with increasing H_2SO_4 and KNO_3 concentrations. Water soaking at ambient temperature (25 °C) and GA_3 treatments did not significantly enhance the FGP, while mechanical scarification had a significantly higher FGP than the control. Scarification increases the germination rate of a number of palm species, indicating that seed coat impermeability to water and/or gases may be involved (Al-Salih, 1984; Odetola, 1987). In arid and semi-arid species such as *Helianthemum*, mechanical scarification significantly increased germination percentage (Pérez-García and González-Benito, 2006). Soaking intact seeds of *Thrinax* in 97% H_2SO_4 for 30 min had the same effect of scarification to reduce the germination inhibiting effects due to the thickness of pericarp. Similar results have been reported for *Albizia grandibracteata* seeds (Tigabu and Oden, 2001) and *Areca triandra* seeds (Yang et al., 2007). These indicate that *Thrinax* seeds have exogenous dormancy, and this dormancy is caused by the hard pericarp. The germination speed (GRI and CGRI) and GT_{50} of the intact seeds were significant in the both H_2SO_4 treatments (15 min) for *Sabal* and (30 min) for *Thrinax* and seeds reached 50% of its final germination (GT_{50}) in a minimum time (3.11 and 5.19 days, respectively. These results take the same trend with Shaik et al. (2008) on *Sutherlandia frutescens* and contrasted with Yang et al. (2007) on *Areca triandra*. Germination rate for seeds soaked in water (24 h) was slightly higher than the control of *Sabal* while the *Thrinax* was less than the control. The germination rate of the mechanical scarified seeds of *Sabal* and *Thrinax* was higher than the control and they germinated significantly faster than intact seeds. Compared with the control, 1% KNO_3 had a significantly higher GRI and CGRI of *Sabal* but other concentrations did not significantly affect GRI. In *Thrinax*, the germination rate of 4% KNO_3 and control was identical, but the other concentrations were significantly lower than control. The germination speed of *Sabal* increased with increasing GA_3 concentration however, in *Thrinax* it was significantly lower than control. GT_{50} of water soaked (24 h) was achieved after 9.5 and 12.5 days of *Sabal* and *Thrinax*, respectively but H_2SO_4 treatments of two plants achieved the GT_{50} after a few days when compared with control. The mechanically scarified seeds reached to GT_{50} after 4.3 and 7.4 for *Sabal* and *Thrinax*, respectively. Yang et al. (2007) reported that mechanical scarification accelerates germinaition and some chemical treatments significantly increased germination speed of the mechanically scarified seeds. Sharif-Zadeh and Murdoch (2001) reported that scarification improves seed germination, but it is an inconvenient method and also produces inconsistent results. The highest final germination percentage for *Sabal* was recorded 95% for both water soaking (24 h) and GA_3 at 500 ppm while for *Thrinax*, it was recorded 85% and 90% at mechanical scarification and H_2SO_4 for 30 min, respectively. The present investigation demonstrates that *Thrinax* seeds exhibit exogenous dormancy which is entirely imposed by the hard seed coat, whereas the *Sabal* seeds exhibit both exogenous and physiological dormancy.

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