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EFFECTS OF DIFFERENT METHODS OF BREAKING DORMANCY AND SEED GERMINATION RATE IN DATE PALM (*Phoenix dactylifera* L.)

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ABSTRACT

Seed dormancy in palms, date palm inclusive have continued to be challenging especially when cultivating through seeds. This study evaluated different methods of breaking dormancy and seed germination rate in P. dactylifera. Using Completely Randomize Design, two varieties of P. dactylifera were treated with three treatments comprising sulphuric acid, hot water, cold water and control. Result showed that all the three treatments significantly ($p < 0.05$) affect the germination rate and early seedling growth of P. dactylifera. From the results, acid treatments had the highest (10) effect on the germination rate and early seedling growth of P. dactylifera, followed by cold water (6), hot water (2) and control (1), respectively. Furthermore, results found all the treatments to have no significant ($p>0.05$) effect on seedling growth parameters (fresh and dry) weight. However, seed treated with sulphuric acid had less weight, compared to other three treatments. This study demonstrates that seed dormancy in P. dactylifera can best be overcomed by immersing the seeds in concentrated sulphuric acid for three minutes. However, soaking in hot or cold water for five minutes for 24 hours may yield fruitful results. Application of hot and cold water to overcome P. dactylifera seed dormancy is crucial to minimize the cost of production using sulphuric acid. It is recommended that in depth research should be conducted to find out if the use of sulphuric acid could affect the DNA sequence of P. dactylifera seed.

Key words: Dormancy, germination, *phoenix dactylifera*, seed, seedling growth.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a monocotyledonous plant belonging to the family Arecaceae which contains 200 genera and more than 2000 species (Diaz *et al.*, 2003). It is suggested that date palm originated from Mesopotamia since 4000 BC, and by the Egyptians since 2000-3000 BC (Manickavasagan *et al.*, 2012). Date palm is considered the oldest fruit tree in the world (Zaid and De Wet, 1999), and is distributed in tropical and subtropical regions where it has been cultivated in North Africa and the Middle East for millennia. Although more than 2000 cultivars of date palm are found worldwide, but few are promising in agriculture (Mrabet *et al.*, 2008). Dates play a vital role in the socioeconomic well-being of the people living in the aforementioned regions and elsewhere. In addition, date palm play instrumental role in their daily diet and in treatment of several diseases, e.g. treatment of abdominal problems, ulcer, inflammation, etc. (Sulieman *et al.*, 2012).

Seed dormancy is defined as nature's way of setting a time clock that allows seeds to initiate germination when conditions are suitable for germination and establishment of the seedlings (Baskin and Baskin, 2004a). In other words, it is an innate seed property that defines the environmental conditions in which the seed is able to germinate, whereas viable seeds that do not germinate are said to be dormant. On the other hand, dormancy is a mechanism used by plants to prevent germination during unfavorable conditions, that is, when the probability of seedling survival is very low (Black and Halmer, 2006). Different mechanisms used to break dormancy in nature include physical rubbing of the seed coat to make it thinner so that water and gases can diffuse into seed, change and fluctuation of atmospheric temperature, seeds passing through the guts of animals and birds, long wet and or frosty conditions and fire (Baskin and Baskin, 2004b). However, some artificial methods used to break

dormancy are scarification, treatment of seeds with chemicals like acids and soaking of seeds in water.

Despite the tremendous benefits that can be derived from growing *P. dactylifera,* its cultivation in many parts of the world, e.g; Nigeria, continues to be a challenge because of the difficulty of its establishment using seeds. Amy (2010) reported that the record of germination success of most palm seeds is poor, while viable seeds can germinate between 14 and 21 days under ideal conditions, whereas healthy seeds may take up to 100 days to germinate because of dormancy problems. Consequently, some studies investigated seed germination and dormancy breaking methods of wild date palm (von Fintel *et al.,* 2004) and other related palms (Green *et al.,* 2013). Nonetheless, available studies on *P. dactylifera* were geared towards its proximate analyses, i.e. phytochemicals screening, nutritional properties, medicinal benefits. However, little attention is given to how dormancy affects date palm seed. This study aimed to evaluate the efficacy of different methods of breaking dormancy on seed germination and early seedling growth of date palm (*Phoenix dactylifera*). Information provided by this study will help in enhancing seed germination and promoting seedling growth arising from date palm seed dormancy.

MATERIALS AND METHOD

Study Area

The study was conducted between May and August, 2017 at the Laboratory of Biological Sciences Department, Federal University Dutse located between Latitude 11.42' ’31.8N and Longitude 009°22’16.0E. The area is semi-arid environment characterized by Sudan and Guinea Savannah vegetation. Rainfall in the region is unimodal starting from May to September, and a dry season starting from October through April. The average temperature is around 30.9° C during the hottest period, and 22° C during the coldest period, January (*https://en.climate-data.org/location/46667/*; *https://en.m.wikipedia.org/wiki/Dutse*). Dutse is home to one of the five substations of the Nigerian Institute for Oil Palm Research (NIFOR), with an aim to improving genetic date palm varieties, collection and conserving gene pool of date palm varieties for breeding purposes, as well as production of the seeds with desired characteristics. Thus, these goals have great potential for economic mass Date palm production and seedling distribution.

Methodology

Sample Collection and Laboratory Procedure

Two varieties of date palm seeds with substation tag number P5R3GPV and P2R1GPIII were procured from NIFOR, and were germinated in the laboratory. The research approach was Completely Randomized Design with two replications. A method of breaking seed dormancy described by Al-Fredan and Ali (2008) was adopted. This method includes soaking of seeds in either acid, cold water, or hot water. The treatments comprised four combinations: (i) Acid treatment (20 seeds of each variety were soaked in concentrated sulphuric acid (H_2SO_4) in a beaker with gentle stirring using a glass rod for three minutes), (ii) Cold water treatment (20 seeds of each variety were soaked in cold water in a beaker for 24 hours, (iii) Hot water treatment (20 seeds of each variety were soaked in hot water for five minutes in a beaker with gentle stirring using a glass rod for five minutes), and (iv) Control (untreated seeds). The reason for the time variations among the different treatments was due to the nature of the treatments. After completion of all of the treatments, the seeds were soaked in water in plastic containers for three days (this was to allow the seeds to imbibe sufficient water in order to activate the enzymes for the germination process), after which the water was discarded, seeds were then washed everyday for 11 days to prevent drying. Number of germinated seeds and growth parameters (fresh and dry) weight were taken for the two varieties in all the treatment combinations.

Determination of Germination Rate

Germination was observed and recorded at three-day intervals, and germination rate was computed according to Okunlola *et al.* (2011) using the following formula:

$$\text{Germination rate (\%)} = \frac{\text{Number of germinated seeds at time, t}}{\text{Number of soaked seeds at time, t}} \times 100$$

Determination of Fresh and Dry Weight of *P. dactylifera* Seedlings

After successful germination of the seeds, five seedlings of each variety were selected randomly from all the treatment combinations. Their fresh weights were measured using an electronic balance, then spread to air dry for 12 days, within which dry weight was measured three times at four-days

intervals. Recording of the dry weight mass was terminated at the third record because the seedlings did not show further significant reduction in weight.

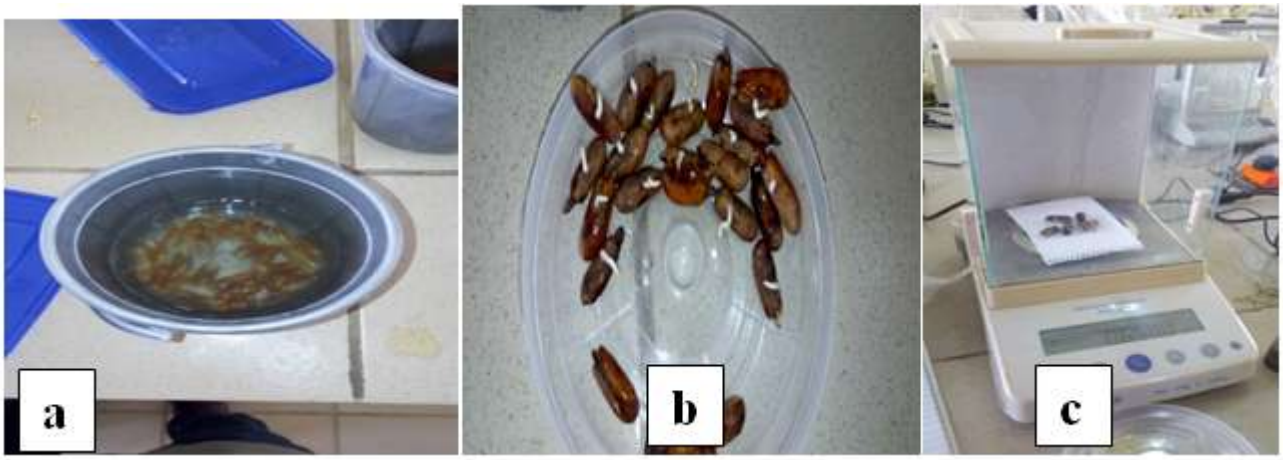
Data Analysis

Mean germination rates, fresh and dry weight were analyzed using two-way ANOVA, while Least Significant Difference was used to compare between the means, i. e. average of the treatment replications. Genstat statistical package (Pyne *et al.*, 2009) was used for the analyses. The level of significance was set at $p<0.05$.

RESULTS

Mean Germination Rate of *P. dactylifera* Seeds

Results from this study indicated that the different methods of breaking seed dormancy significantly ($p<0.05$) affected the germination rate and early seedling growth of *P. dactylifera* (Figure 1). At five days after soaking, it was observed that acid treatment had the highest (10) influence on the germination rate of the seeds among all the treatment combinations, followed by cold water (6), hot water (2), and then control (1) and the difference was significant ($p< 0.05$). After eight days of soaking, however, acid and cold water treatments did not differ significantly ($p>0.05$). Similarly, hot water treatment did not differ significantly from the control. From the results, at 11 days after soaking, acid and cold water treatments had the highest mean germination rate, followed by hot water and then control. Seeds in the control had the lowest mean germination rate, compared to other three treatments and the difference was significant ($p<0.05$).



Plates 1: (a) Seeds soaked in cold water during the laboratory work,
(b) Germinated *P. dactylifera* seeds, and
(c) Weighing *P. dactylifera* sprouted seeds.

Table 1: Mean germination of the two *P. dactylifera* varieties recorded in different treatments

	Mean germination rates (DAS)			
Treatments	5DAS	8DAS	11DAS	14DAS
Acid	10^a	14^a	15a	15^a
Hot water	2^c	6^b	12b	16a
Cold water	6^b	12^a	15^a	14a
Control	2^c	5^b	7^c	9^b
LSD	2.91	2.70	2.48	2.38
Variety				
V_1	7^a	13^a	15^a	17^a
V_2	3^b	5^b	9^b	10^b
LSD	2.06	1.91	1.75	1.68
Interaction				
Trt*Var	NS	NS	NS	NS

Values within columns followed by the same letter were not significantly different at $P<0.05$.Key: (DAS) Days After Soaking, (LSD) Least Significant Difference, (NS) Not Significant, (Trt) Treatment, Variety One (V_1), Variety Two (V_2), (Var) Variety.

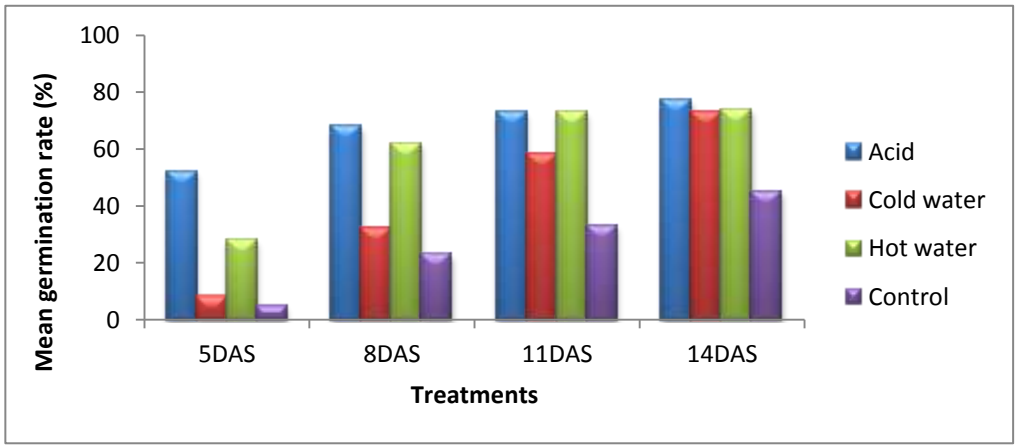


Figure 1: Mean germination rate of the two *P. dactylifera* seeds recorded in different treatment conditions.
Key:
DAS = Days After Soaking

Similarly, findings of the present study showed that all the methods used in breaking seed dormancy had an effect on the germination and early seedling growth of *P. dactylifera* when analyzed for different varieties. It was observed that variety V_1 had significantly ($p<0.05$) higher germination rate across all the recording days interval compared to V_2 variety (Figure 2), and the difference between all the means were greater than the Least Significant Difference value.

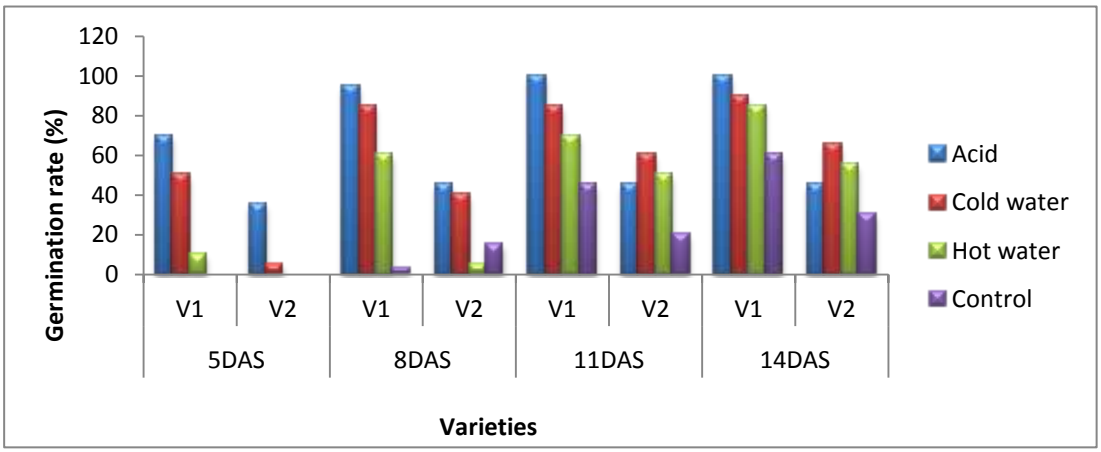


Figure 2: Mean germination rate of seeds recorded from the two *P. dactylifera* varieties.
Key:
Days After Soaking (DAS).

Mean Dry and Fresh Weights of *P. dactylifera* Seedlings

Results indicated that all the different methods used in breaking seed dormancy in *P. dactylifera* affect the fresh and dry weight of the germinated seedlings (Table 1). At three times of the recorded intervals, the dry weight of germinated seeds treated with acid was significantly ($p<0.05$) lower than all the three treatments. With regard to varieties, the dry weight of V_2 seedlings differs significantly ($p<0.05$) compared to V_1 throughout the recorded time intervals. The fresh weight of the seedlings recorded in acid treatment was 8.45 g, which is lower than 9.37, 9.42 and 9.96 g recorded in the control, hot water, and cold water treatments, respectively. Although the fresh weight did not differ significantly ($p>0.05$) from each other with regards to the treatment combinations, there were differences between varieties: V_1 had significantly ($p<0.05$) lower fresh weight than V_2.

Table 2: Mean dry and fresh weights (g) of *P. dactylifera* seedlings

	Dry weight (g)			
Treatments	**4DAD**	**8DAD**	**12DAD**	**Fresh weight (g)**
Acid	5.47[b]	5.39[c]	4.85[b]	8.45
Hot water	6.22[a]	5.79[b]	5.73[a]	9.42
Cold water	6.58[a]	6.19[a]	6.17[a]	9.96
Control	6.25[a]	6.10[a]	6.04[a]	9.37
LSD	0.84	0.30	0.59	2.2
Varieties				
V_1	5.47[b]	5.11[b]	5.09[b]	8.45[b]
V_2	6.79[a]	6.62[a]	6.30[a]	10.15[a]
LSD	0.59	0.35	0.42	1.53
Interaction				
Trt*Var	NS	NS	NS	NS

Values within columns followed by the same letter were not significantly different $P>0.05$.

Key:
DAD = Days after drying, LSD, = Least Significant Difference, NS = Not Significant, Trt = Treatment, V_1 =Variety One, V_2 = Variety Two (V_2), and Var, = Variety.

DISCUSSION

Germination Rate of *P. dactylifera* Seeds

This study demonstrates the effectiveness of various methods of breaking dormancy on *P. dactylifera* seeds. It also shows how seeds responded to the different treatment combinstions. Results indicated that seed dormancy in *P. dactylifera* arises from the hardiness of the seed coat. This finding is similar to that of Okunlola *et al.* (2011) who reported that seed dormancy in *Parkia biglobosa* was associated with the hardness of the seed coat. The increase in the germination rate of the date palm seeds could be attributed to the removal of the cuticle and softening of the seed coat by the different methods tested for breaking seed dormancy of *P. dactylifera*. Treatment of seeds with concentrated sulphuric acid was found to induce the highest germination rate compared to the other three treatments. This could largely be attributed to the influence of the acid that acted on the seed coat. This also may have penetrated the seed coat and inhibited the chemical compounds causing the dormancy. This observation concurs with other studies (Northcutt *et al.*, 2012; Purohit, 2015).

Similarly in overall, this study suggests that the reason for the high germination rate in hot water might be as a result of the action of the hot water on the seed coat and also on the chemical constituents. Further possible explanation for the high germination rate in hot water compared to the other three treatments was likely due to the high temperature of the hot water. This observation is in line with other studies (Al-Fredan and Ali, 2008; Dewir e*t al.*, 2011) who observed hot water increased the rate of germination by its action on the seed coat, consequently breaking down the bonds between the chemical compounds in the seed coat responsible for causing dormancy. On the other hand, seeds treated with cold water showed progressive germination rate throughout the days after soaking. This is probably because the germination rate of the seeds was determined by how long the seeds are soaked in cold water considering the fact that the temperature of the cold water was low, compared to the action of acid and hot water. Similar finding was reported by Vleeshouwers *et al.* (1995). However, at 14 days after soaking, it was noted that nearly all seeds in all the treatment conditions showed more than 75% germination rate except the control (which showed 45%), confirming the effectiveness of all the three methods in breaking seed dormancy in *P. dactylifera*. With respect to varieties, V_1 was observed to respond more to the different treatments, compared to V_2 throughout the subsequent days after soaking. The probable explanation for this might be V_1 has a shorter dormancy period, compared to V_2 (Bewley and Black, 2012). In addition, from a physiological perspective, there could be differences in the embryonic development stages between V_1 and V_2. Thus, V_2 might have had an underdeveloped embryo compared to V_1.

Fresh and Dry Weights of *P. dactylifera* Seedlings

The study revealed that all the treatments had an effect on seedling growth parameters (fresh and dry weight) of *P. dactylifera*. However, seedlings treated with acid had less weight compared to all other three treatments. This is not surprising because as stated earlier, acid treatment had the greatest influence on the seed germination rate. This

facilitated early germination rate could have caused the seeds soaked in acid to lose their stored food reserve (food substances) in the endosperm. This agrees with Nedeva and Nikolova (1999) who described that as a seed develops, it uses up the reserve food material stored in the endosperm. Regarding varieties, V_1 seeds germinated faster than V_2 probably due to the faster consumption of the stored fats in V_1, compared to V_2.

Results further showed that the measured fresh weights were higher than the dry weight, probably because of the imbibed water together with the dry matter contents of the seedlings. The increased mass recorded in fresh weight could be not only as a result of the accumulation of dry weight, but because of the facilitation of water imbibitions by the different methods of breaking seed dormancy. This is in support of Nedeva and Nikolova (1999) who observed fresh weight of wheat seed was doubled in weight due to the simultaneous accumulation of dry weight and water uptake. Regarding the dry weight, findings showed that the dry weight decreased gradually as the seedlings were getting dryer before halting at a fixed point. This probably determined the actual weight of the dry matter of the seedlings. The decrease in dry weight compared to fresh weight of the seedlings observed in this study could be due to the loss of water content because as the seed dried up, the developing embryo utilized the reserve substances in the endosperm in the course of seedling establishment (Bewley and Black, 2012).

Conclusions

The findings of this study indicated that dormancy in date palm arises from the hardness of the seed coat. Thus, the need to overcome it is critical to aid water and gas exchange to enhance the rate of germination of the date palm seed. Overall, it is concluded that:

i. Dormancy in date palm seeds can best be overcome by immersing the seeds in concentrated sulphuric acid for three minutes. Nonetheless, some other methods, specifically the use of hot water also helped to induce the seed germination rate. In addition, as observed, soaking the seeds in hot water for five minutes or in cold water for 24 hours can also yield a good germination rate.

ii. This study showed that the different methods of breaking seed dormancy were observed to have an effect on the growth parameters, i.e. fresh and dry weight of the seedlings.

Recommendations

It is recommended that:

i. Further studies should be carried out to observe the effect of the different methods of breaking seed dormancy on the seedling growth of date palm, such as seedling vigor.

ii. The experimental methods used in this study should be tried in the natural environment (field) to observe the influence of different soil types on the germination rate of date palm seedlings.

iii. At genetic and molecular levels, in depth research should also be conducted to find out if the use of sulphuric acid could change the DNA sequence of the *P. dactylifera* which may possibly cause mutation in the form of chemical mutagen.

iv. Application of hot and cold to overcome *P. dactylifera* seed dormancy is crucial especially for local farmers, and large-scale date palm cultivators, to minimize cost of production using acid where it is adequately not accessible.

Conflict of interest

The authors declare that they have no conflicts of interest in this research work.

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