

Effect of stratification and sulphuric acid scarification for breaking seed dormancy on different genotypes of Arecaceae family; Phoenix dactylifera and Washingtonia robusta.

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Abstract

This study aims at examining the effect of stratification and sulphuric acid scarification treatments on Medjool, wild date palm (*Phoenix dactylifera*) and *Washingtonia robusta* seeds. The results showed that the highest germination percentage was obtained from wild date palm genotype. The results also showed that the highest plumule length was obtained from *Washingtonia robusta* seed after 30 days of planting. Furthermore, the study showed that the employment of different pre sowing treatments induced significant increase in germination percentage, radicle length and plumule length of seeds after 20 and 30 days of planting. The highest germination percentage was reported when the seeds were immersed in cold water (8 °C) for 12 hours followed by hot water (80 °C) for 8 hours. Additionally, it was found that the type of water source during germination process (whether tap or distilled water) had no significant effect on germination percentage or radicle and plumule lengths.

Keywords: Germination percentage; Radicle length; Plumule length; Scarification; Stratification.

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تأثير معاملات التنضيد والتخديش باستخدام حمض الكبريتيك لكسر سكون البذور على طرز

جينية مختلفة من العائلة النخيلية

Phoenix dactylifera* and *Washingtonia robusta

معاوية عايد العساسفة

ملخص

تهدف هذه الدراسة إلى دراسة تأثير التنضيد والتخديش باستخدام حمض الكبريتيك على طرز جينية مختلفة من العائلة النخيلية مثل: صنف المجهول ونخيل التمر البري *Phoenix dactylifera* وبذور *Washingtonia robusta*. أظهرت النتائج أن أعلى نسبة إنبات كانت للطرز الجينية من نخيل التمر البري. وأظهرت النتائج أيضاً أنه تم الحصول على أعلى طول رويشة في بذور *Washingtonia robusta* بعد 30 يوماً من الزراعة. علاوة على ذلك ، أوضحت الدراسة أن استخدام المعاملات المختلفة قبل الزراعة لكسر السكون تساهم في زيادة كبيرة في نسبة الإنبات ، وفي أطوال الرويشة والجذير بعد 20 و 30 يوماً من الزراعة. ومن الجدير بالذكر أن أعلى نسبة إنبات للطرز الجينية المختلفة كانت عند غمر البذور في الماء البارد (8 درجات مئوية) لمدة 12 ساعة متبوعة بالماء الساخن (80 درجة مئوية) لمدة 8 ساعات. بالإضافة إلى ذلك ، لم يكن لمصدر المياه أثناء عملية الإنبات (سواء كان من الصنبور أو باستخدام الماء المقطر) أي تأثير معنوي على نسبة الإنبات أو أطوال الرويشة والجذير.

1. Introduction

The Arecaceae (Palmae) family varies in propagation methods and each genus has at least one propagation method that is successful and effective (Zaid & De Wet, 2002). For example, date palm (*Phoenix dactylifera* L.) could be propagated by different methods including seeds, offshoots or tissue culture, while *Washingtonia* genus is propagated only by seeds. Trees propagated by seeds have high genetic variation and have a longer juvenile stage than those propagated by other methods (Zaid & De Wet, 2002). It was also noticed that the seed propagation (sexual propagation) is not a preferred method of date palm propagation. However, it might be useful for breeding purposes, ornamental and feeding. Seed propagation is also considered the easiest and fastest method for propagation. Planting date seeds is the most economical way to select clones that have desirable characters such as pest and salt tolerance (Meerow, 1990).

Dormancy is a condition where seeds will not germinate even when the environmental conditions such as water availability and temperature are favorable for germination. It is determined by genetics, environmental influence and plant hormones (Mayer & Poljakoff-Mayber, 1989). In physiological dormancy, the tissues surrounding the embryo can affect germination by inhibiting water uptake, providing mechanical resistance to embryo expansion and radicle emergence, limiting oxygen gas exchange of the embryo, or by regulating embryo inhibitor supply. However, it is an efficient mechanism to help seeds' survival and spread. It is also evident that physical dormancy is caused by impermeability of water and gas to embryo by the cell layers of seed coat (Baskin & Baskin, 2004). Generally, germination of untreated palm seeds with hard, solid and inflexible seed coat will be slow (Meerow, 1990).

Germination percentage can be improved through some pre-sowing techniques (Azad et al., 2006a, 2006b, 2011). Many pre-sowing treatments of seed germination were tested to break seed dormancy and increase the germination rate. Scarification of seed coat may overcome seed dormancy (Catalan & Macchiavelli, 1991). Acid treatment and hot water treatment (Mafatlal & Nataraj, 2015; Airi et al., 2009; Kobmoo and Hellum, 1984) are used to break seed dormancy as well.

Many studies have shown that weakening the seed coat either by mechanical, chemical scarification or soaking in cold/hot water might enhance the permeability of water and gases to the embryo (Vleeshouwers

et al., 1995). Okunlola et al., (2011) noticed that the cold/hot water treatment of some forest trees seeds (*Parkia biglobosa*) increases germination rate and seedling height. Meerow (1990), on the other hand, argued that pre-sowing treatment of palm seeds by immersing the seeds in water can also improve the germination percentage. He also noticed that the water temperature (30–35°C) can play a significant role in increasing seed germination percentages. (Habila et al., 2016) indicated that the boiling water had the most effective method of breaking date palm seed dormancy. (Muhammad et al., 2017) & (Kheloufi et al., 2017) showed that acid treatments had the highest effect on germination rate and seedling growth of *P. dactylifera* and three other species of *Acacia* respectively. A significantly high germination percentage for *Grewia damine* Gaertn seeds was recorded in the treatment of scarification with a sand paper (De Mel & Yakandawala, 2016).

Phoenix dactylifera and *Washingtonia*. sp are important trees species for agroforestry, social forestry, home garden, rapid growth and tolerance for infertile and dry seasons. Those special traits make them very useful species for rehabilitation of degraded lands. Therefore, these species are considered a fertile ground for researchers especially when it comes to studying the appropriate seed germination techniques.

The current study examined the effect of pretreatment methods of breaking seed dormancy on two genera of Arecaceae (*Phoenix dactylifera* and *Washingtonia* sp). Medjool cultivar, wild date palm from *Phoenix dactylifera* and *Washingtonia robusta* (from *Washingtonia* genus) are wild date palm that distributed in dry Middle Eastern regions as well as north Arabian deserts. It is closely related to the cultivated date palms. Moreover, it shows morphological similarities, climatic requirements and production of the basal suckers therefore botanists place it under *P. dactylifera* L. (Jaradat, 2011). It is characterized by having small fruits containing relatively little edible flesh and dark brown to dark grey bark. It also occurs in a dense stand that does not grow much more than cultivars in height and the leaves are roughly scarred (Alasasfa et al., 2015).

Only very scarce studies examined the breaking of seed dormancy of Arecaceae family in Jordan. The current study attempts to examine the effect of different dormancy breaking pretreatment on three genotypes: Wild

date palm, Medjool and *Washingtonia robusta*. The study also attempts to investigate the effect of such breaking pretreatment on the seed response, seed germination and early seedling growth of the three genotypes under investigation.

2. Materials and Methods

2.1. Experimental site

The experiment was carried out in the Department of Plant production, Faculty of agriculture, Mutah University, Jordan.

2.2. Source of materials

Three genotypes were comprised in this study (wild, Medjool and *Washingtonia* genus). The wild date palm seeds were collected from Wadi Ibn Hammad, Karak governorate, Jordan; Medjool seeds were collected from Ghour Alsafi, Karak governorate, Jordan and *Washingtonia* seeds were collected from Faculty of Agriculture in Mutah University. The collected seeds were washed with fresh water three times to remove the remaining fruit pieces. The seeds were dried for 6 to 8 days in aerated and shaded area. Then, the seeds were kept in a dry place for ten days to reduce the moisture after collection. Only healthy dried seeds were used for the experiment (Figure 1). The seeds were exposed to seven pre-planting treatments and control, with two water sources for planting: tap water (W1) and distilled water (W2) for each treatment. The treatments used for seeds are given as follows:

2.3. Treatment keys

Treatment 1 (T1): Control; seeds were planted without any pre planting treatments.

Treatment 2 (T2): Immersion in normal water at room temperature (20–22°C) for 12 hours.

Treatment 3 (T3): Immersion in warm water (25–35°C) for 12 hours.

Treatment 4 (T4): Immersion in hot water (80°C) for 8 hours.

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Treatment 5 (T5): Immersion in cold water (8°C) for 12 hours.

Treatment 6 (T6): Immersion in hot then in cold water (80 °C \ 8 °C) for 8 and 12 hour ss(respectively).

Treatment 7 (T7): Immersion in cold then in hot water (8 °C \ 80 °C) for 12 and 8 hours (respectively).

Treatment 8 (T8): Immersion in concentrated H₂SO₄ for 4 minutes.

2.4. Treatment application and planting of seeds

The germination test was conducted in December 2017 by sowing 256 seeds from each source on cotton in paper cups (12 Oz). The seeds were distributed in eight treatments. Each treatment had 32 seeds. Half of them were irrigated with tap water and the rest were irrigated with distilled water (two seeds in a paper cup with eight replications with the two source of watering, for eight treatments; 2*8*2*8). They were covered by poly bags. Poly bag paper cups were kept in growth chamber throughout the experiment at 27 °C under dark conditions. Measurements were recorded twice during the experiment (i.e. after 20 and 30 days of planting). Data were analyzed using variance test (ANOVA), SAS version 9.0 (Statistical analysis System) (2002) software. The means were separated with the least significant difference (LSD) for comparison of means, under 0.05 % probability level.

2.5. Data Collection and analysis

2.5.1. Germination percentage (%)

The germination percentage was calculated as the ratio of germinated seeds to the total number of seeds planted (Okunlola et al., 2011) at 20 and 30 days after planting (DAP).

$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated seeds}}{\text{Number of planted seeds}} \times 100$$

2.5.2 Radicle and plumule length

The radicle and Plumule lengths of the seedling sampled were measured using measuring ruler.



Fig(1) Seed genotypes of Arecaceae family: A. Medjool seeds (*P. dactylifera*). B. Wild date palm seeds (*P. dactylifera*) and C. (*W. robusta*) seeds.

3. Results

3.1 Seed genotype effects

3.1.1 Seed germination

Seed germination started when radicle formed (Figure 2). Results showed that seed type has a key role in germination percentage either after 20 or 30 days of seeding.



Fig(2) The germination begins as soon as the radicle appears: A. Medjool date palm. B. Wild date palm and C. (*W. robusta*).

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Results showed that the highest germination percentages (79.56% and 86.65 % after 20 and 30 days of seeding respectively) were obtained by wild date palm seed while the lowest percentages (23.12% and 26.25% after 20 and 30 day of planting, respectively) were obtained by Washingtonia seeds. With regards to ‘Medjool seeds’, the germination percentage were 59.29% and 65.62% after 20 and 30 days of planting. The percentage was not significant from wild date palm but significant from Washingtonia seeds as shown in Table (1). The germination of seeds treated with immersion in cold water for 12 h and hot water for 8 h (87.5%) was significantly compared to the control.

Table (1) Effect of seed genotypes on germination percentage, radicle length and plumule length after 20 and 30 days of planting.

	Germination percentage		Radicle length (cm)		Plumule length (cm)	
	20 days	30 days	20 days	30 days	20 days	30 days
Wild date palm	79.56 a	86.65 a	2.59 a	5.1 a	0 b	0.37 b
Medjool	59.29 a	65.62 a	1.23 b	3.08 b	0 b	0.09 b
Washingtonia sp.	23.12 b	26.25 b	0.84 b	1.18 b	0.7 a	1.25 a

*** Values within same column that have different letters are significantly different at 0.05 level of probability according to LSD.**

3.1.2. Radicle length

Results in Table (1) clearly show that radicle length was significantly affected by seed type. The highest radicle length was obtained from wild date palm (2.59 and 5.1cm after 20 and 30 days of planting, respectively) and the lowest one was reported from Washingtonia seeds. Results showed that radicle length had significant differences between wild date palm and other seeds. However, no significant differences were reported between Medjool and Washingtonia seeds as shown in Table (1).

3.1.3. Plumule length

Plumule length was also significantly affected by seed type as shown in Table (1). The highest plumule lengths (0.69 cm and 1.25 cm after 20 and 30 days of planting, respectively) were obtained by washingtonia seeds and the lowest one (0.09 cm) was obtained from Medjool seeds. The statistical analysis, however, showed that there was no significant difference in plumule length of 'wild date palm and 'Medjool' seeds.

3.2. Treatment effects

3.2.1. Germination percentage

Germination started significantly earlier in all pretreatment compared to the control (T1). Seed treated with cold / hot water (T7) germinated significantly higher (87.5%) than that of T1 (0%) and T2 (51%). Germination percentage was also significantly higher (75%) in cold water (T5) after 30 days of planting than that of T1 (Table 2). Least significant differences showed significant difference on seed germination in T7 with control and immersion in water at room temperature (T2). Also, T5 has significant differences with T1, but there was no significant difference with T2, T3, T4, T6, T7 and T8 (See Table 2).

3.2.2. Radicle length

Results showed that there were significant differences among the treatments related to radicle length after 20 and 30 days of planting (Table 2). The highest radicle length (6.33 cm) was obtained by hot temperature (T4). But the lowest radicle length (0.02 cm) was obtained by control treatment (T1).

3.2.3. Plumule length

The results in Table (2) clearly show that the plumule length was also affected by the pretreatments. The highest mean plumule length (1.92 cm) was obtained from hot temperature treatment (T4) after 30 days of planting.

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The lowest mean length (0 cm) was obtained by T1, T2 and T3 pretreatments, which could be related to the delayed seeds germination process. There were significant differences in plumule length between (T4) treatment with T1, T2, T3, T5 and T8, while the other treatments have no significant differences among them.

Table (2) Effect of different pretreatments on germination percentage, radicle length and plumule length after 20 and 30 days of planting.

Pretreatment	Germination percentage		Radicle length (cm)		Plumule length (cm)	
	20 days	30 days	20 days	30 days	20 days	30 days
T1	0 c	10 c	0 d	0.02 d	0 b	0 b
T2	50.83 bc	55 bc	0.6 cd	2.26 cd	0 b	0 b
T3	54.2 ab	58.33 ab	0.07 d	1.72 cd	0 b	0 b
T4	74.17 a	74.17 ab	4.1 a	6.33 a	1.12 a	1.92 a
T5	61.11ab	75 ab	2.01 bc	4.39 abc	0 b	0.4 b
T6	70.83 ab	70.83 ab	1.82 bc	3.49 abc	0.25 b	0.75 ab
T7	87.5 a	87.5 a	2.92 ab	5.2 abc	0.42 ab	1.34 ab
T8	53.99 ab	59.51 ab	1.56 cd	3.12 bcd	0.22 b	0.57 b

* Values within same column that have different letters are significantly different at 0.05 level of probability according to LSD.

3.3. Water source effects

In all water sources, germination started after 20 days of planting (Table 3). LSD showed no significance difference ($P>0.05$) in germination.

Results in Table (3) indicated that water source has not effect on germination percentage, radicle length and plumule length. It is worth noting that high germination percentage (59.6 %, Table 3) was recorded in distilled water treatment after 30 days of planting.

Table (3) Effect of water source on germination percentage, radicle and plumule length after 20 and 30 days of planting.

Water source	Germination percentage		Radicle length (cm)		Plumule length (cm)	
	20 days	30 days	20 days	30 days	20 days	30 days
Tap water	52.65 a	59.42 a	1.64 a	3.13 a	0.15 a	0.55 a
Distilled water	55.33 a	59.6 a	1.47 a	3.11 a	0.29 a	0.58 a

* Values within same column that have different letters are significantly different at 0.05 level of probability according to LSD.

4. Discussion

The study showed differences in the response of palm seeds to the pretreatments of breaking dormancy. Despite the fact that wild, Medjool, and Washingtonia seeds responded positively to the pretreatments, the wild date seeds performed better. This may be due to adaptation to environmental conditions, high vitality embryo, and that the seed coat of wild date is thinner than its counterparts. The current results of the study are consistent with many other studies in the literature. For instance, the results reported in Kitzke (1958) study showed that the germination percentage of presoaking seed palm can be improved and therefore responded positively. However, this was not true for all species in the same pattern. Also, the current results are hand in hand with the results of Green et al., (2013). In his study, Green et. al. indicated that there were differences in germination percentage and period within six oil palm cultivars. This may be due to the genetic role in the process of breaking seed dormancy.

The results also indicated that Washingtonia seeds showed a greater ability to grow the plumule faster than Phoenix sp. I argue that the reason for this may be due to genetic makeup or to the small size of seeds compared to the others. I further argue that the increase in germination percentage, radicle and plumule length of palm seeds with different pretreatments could be attributed to the weakened seed coat. Consequently, the results were quite similar to those reported in Von Fintel et al., (2004). In this study, Fintel et al. investigated the effect of seed germination and breaking dormancy methods on wild date palm. It was reported that the pretreatment by soaking or acid scarification had no positively significant

effect on wild dates while the application of boiling water was reported to be extremely negative for seed germination process. Kitzke (1958) reported that the duration of the presoak period on seeds of the same species could be varying. He has found that the concentrated H_2SO_4 for 4 minutes will record significantly high germination speed compared to control. Okunlola et al., (2011), however, reported that immersion of seeds of *Parkia biglobosa* in H_2SO_4 for 5 minutes could damage the embryo and lead to insufficient break dormancy.

In the present study, the influence of water source on seed germination, radicle and plumule length was also tested. The results showed that there was no significant effect of water source on the study parameters during the experiment period. Perhaps different water sources could be important only in the advanced seedling stages because of the presence of salt water. This may be due to the fact that the need of seeds for nutrients during early germination stage is very little.

5. Conclusion

Presoaking treatments for “*Phoenix dactylifera*” and “*Washingtonia robusta*” are quite simple and inexpensive for the small-scale farmers and nurseries. In addition, seed dormancy can vary from species to species, stage of maturity of seed and degree of drought. Therefore, pretreatment should be adjusted accordingly. However, further research is needed to investigate the effect of nitric oxide, X-ray and other treatment of breaking seed dormancy in date palm planting.

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