

Effect of mechanical and chemical scarifications of date palm seeds (*Phoenix dactylifera* L.) on *in vitro* germination

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Abstract

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Hard seed coat structure is the main reason for dormancy of Date Palm seed (*Phoenix dactylifera* L.), making it hard to absorb water during germination. Physical, mechanical, or chemical scarification treatments are useful in resolving dormancy conditions in seeds. In the present study, the effect of mechanical (braking and dividing into six groups) and chemical (sulfuric acid (H_2SO_4), potassium nitrate (KNO_3), sodium hydroxide ($NaOH$)) treatments on the germination of date palm seeds was performed for different treatment period (0, 10, 20, 30, 40, 50 min). Among mechanical treatments, intact operculum with scarification showed the highest germination percentage (GP) (86.70%) of the seed *in vitro* germinated. Regarding the chemical treatments, each treatment period had an effect on the seeds, GP of 86.70%, and 80.0% was exhibited for 30 min with $NaOH$ and KNO_3 , respectively. Moreover, soaking of date palm seed in H_2SO_4 for 30 min resulted in the highest GP of 96.7%, on 12th day of culture and number of days lapsed to reach 50% of GP (GT_{50}) of 5.12 days, the germination speed GRI and CGRI of seeds were no significant different in the treatments of H_2SO_4 for 20 or 30 min of the seed *in vitro* germinated. For all treated time, the seeds that pre-germinated *in vitro* shortened the days for germination and resulted in a significant increase in germination percentage compared with soil tested. The results presented herein suggest that date palm seed demonstrated exogenous dormancy that is completely imposed by the hard seed coat. That can be effectively overcome by both mechanical and chemical scarification.

Keywords: mechanical and chemical; scarifications; date palm; seeds; *in vitro* germination, germination percentage

Introduction

Palms are extensively significant to most of the world's population as sources of food and fiber (Ellison and Ellison, 2001). Date palm, *Phoenix dactylifera* L., a monocotyledonous angiosperm of Palmaceae (*Arecaceae*) family, is native to the tropical or subtropical regions of Africa or Southern Asia (Al-Alawi et al., 2017). It is the utmost fruit crops cultivated in arid and semi-arid regions of Middle East, North Africa, and in many Arab countries, such as Saudi Arabia and Iraq (Al-Khayri, 2001). It occupies distinct importance for its renowned economic, nutritional, esthetic, historic and social implications (Khierallah and Bader, 2007).

Palms are amongst woody plants and notorious in the nursery trade for its time-consuming and imbalanced seed germination (Balslev, 1991). The restrictions of this state on the activity of the conventional propagation method include braking and implantation offshoots. There are numerous causes for this behavior: Firstly, Date palm is mainly a diploid, $2n = 2x = 36$, dioecious tree species with separate male and female plants. For fruit setting, fertilization of the female flowers is necessary which regularly includes manual or mechanical pollination. Secondly, the date fruit is a single, oblong, one-seeded berry, comprising of pericarp or fruit skin, fleshy mesocarp, and membranous endocarp around the seed (Al-Khalifah et al., 2012). Thirdly, seed germination of

date palm is relatively slow (Hodel, 1977) as it takes more than a week for the embryo to protrude beyond the seed coat at 27°C (Said, 1989). Belated and uneven seed germination is usual in nursery state. It has been likely that over 25% of all palm species need above 100 days for germinating and they have a lesser amount than 20% total germination (Marcus and Banks, 1999b). The main cause is the presence of certain water soluble growth inhibitors (Ahmed, 1989b),

In fact, scarification increased the germination rate of a number of palm species, signifying that seed coat impermeability to water or gases (Al-Salih, 1984; Odetola, 1987). However, it is not known whether other factors are linked in seed dormancy (Mayer & Poljakoff-Mayber, 1989). The germination in dormant seeds is improved by scarification (physically or chemically altering the seed coverings). It's a horticultural necessity for species with physical dormancy (hard, impermeable seed coats) to permit water uptake.

Scarification of palm seed comprises of thinning the bony endocarp of palm seeds that may inhibit water imbibition. The rate of germination of a number of palm species with hard, water-impermeable seed coats has been enhanced by scarification (Nagao et al., 1980; Odetola, 1987). In mechanical treatment, the surface of the seed was abraded until the endosperm appear visible or the seed is soaked in different concentrations of sulfuric acid for 10 to 30 min (Meerow & Broschat, 1991). The embryo might be injured during the mechanical or acid scarification process. This process should be reserved for seeds with hard and impermeable seeds coats. Species with slow or irregular germination without scarification should have seed scarified on an experimental basis before treating the whole seed lot (Meerow & Broschat, 1991).

Expansion of the germination rate of date palm would be very advantageous as seedlings are employed as sources of explants for *in vitro* tissue culture manipulations (Tisserat, 1979) in addition to short term investigations (Al-Whaibi, 1983; Alsewaigh et al., 1991; Khali et al., 1983). A well-known way for germination improvement is by soaking the seed in water for 1 to 7 days. These pretreatment is beneficial only after dormancy necessities have been met, while few palm species have been examined for indications of seed dormancy (Broschat & Donselman, 1987). The seed must be planted instantly after the treatment. Storage may produce a secondary dormancy as water imbibed during treatment. Water soaking treatment is not responded completely by some species (Broschat & Donselman, 1987; Rauch, 1998). It is normally advised to lessen the hardness of seed coat mechanically or chemically to help hydration (Moussa et al., 1998).

Numerous scarifications based on the use of physical or chemical agents may be used to breakseed dormancy (Basra,

2007), e.g. potassium nitrate (Hartmann et al., 1997; Kevsero Lu, 1993), hydrogen peroxide (Ghildiyal & Sharma, 2005) and sulphuric acid (Keshtkar et al., 2008). While other plants may need physical agents to break their seed dormancy such as hot water (Hermansen et al., 1999) and light and temperature (Savage & Metzger, 2006; Merritt et al., 2007; Verma et al., 2010). The main purpose of this study is to improve the seed germination rate and time of date palm cv. Barni. Mechanical and chemical scarifications were also performed in soil or *in vitro* culture.

Materials and Methods

Fully ripened fruits were hand-picked from date palm cv. Barni trees, grown at Al Ais governor, Al Madinah Munawarah, Saudi Arabia. Seeds were removed manually from the fruits, washed with distilled water thoroughly. Any residues from the pit of the date were removed by cleaning and soaking in sterilized distilled water for 12 hrs, this will also allow the pit to absorb moisture necessary for germination. Once they are cleaned, the stratified stony seeds were exposed to various scarification treatments.

Chemical seed scarification

In the experiment, the effect of chemical seed scarification on germination was examined. In containers, the seeds were placed and submersed in 50 ml solution of 1N H₂SO₄, 1N KNO₃ or 1N NaOH treatments for several soaking periods 10, 20, 30, 40 and 50 min (three repetitions with 10 seeds per replicate). The accumulation of carbon on seed surfaces was avoided by stirring the acid with glass rod (every 2 min) as it might affect with the action of acid. Then, the seeds were rinsed few times with sterilized distilled water. These seeds were germinated together with 10 untreated seeds at 25±2°C.

Polystyrene container filled with mixed sterilized sand, peat moss and perlite were used for seed germination seeds. Or *in vitro* culture, under a laminar airflow cabinet, the seeds were surface-sterilized serially with 70% (v/v) ethyl alcohol (30 s) and 20% Clorox (a.i. 5.25% sodium hypochlorite), and finally cleaned copiously three times. Overnight soaking of the seeds was done in 50 mL of sterile distilled water (SDW). Afterwards, the water was discarded.

Additionally, the Murashige & Skoog (1962) MS medium comprised with 3% sucrose was used to culture the *in vitro* seeds. In respect to this, the pH reading was 5.8. Also, 8 g/L agar was dissolved (microwaved) before the process of autoclaving. Then dispense of 60 mL from each of the 250 mL flask. Media autoclaved at 121°C (20 min). The microshoots were incubated at 24 ± 2°C (16 h). Photoperiod

and photosynthetic photon flux density (PPFD) of $50 \mu\text{mol m}^{-2}\text{S}^{-1}$ was provided by cool white florescent lamps. Numerous types of data were obtained after germinated seeds were counted and discarded every day till no more germination occurred for five consecutive days.

Mechanical seed scarification

The seeds endocarp was broken and divided into six groups (A: Intact operculum (control), B: Partial removed operculum, C: completely removed operculum, D: Intact operculum with scarification, E: Partial removed operculum with scarification, and F: Completely removed operculum with scarification) for mechanical scarification method. Plates filled with a mixture of peat and vermiculite (1:1) was used for stony seeds belonging to six groups for germination. They were kept in a greenhouse at 20 to 25°C or *in vitro* culture. Portions of individual group were than planted aseptically onto MS medium (Murashige and Skoog, 1962) in a sterile flask. Incubations of the cultures are performed in a growth chamber at $25 \pm 2^\circ\text{C}$ (16 hrs light period and light intensity of $50 \mu\text{mol m}^{-2}\text{S}^{-1}$). Germination of *in vitro* culture was decided by the emergence of the radical and opening of the cotyledons.

There are some things to be considered for seed germination. For instance, when the proximal end of the cotyledon sheath had just protruded beyond the seed coat by about 1mm, the seed was considered germinated. Also seed germination percentage and number of days to reach 50% of the final germination (GT_{50}). GT_{50} is used to calculate the seed germination rate (Angus et al., 1980; Hsu et al., 1985; Kanemasu et al., 1975), while CGRI is used for the relative rates of germination comparison (Fulbright & Fulbright, 1990; Hsu et al., 1985). Analysis of variance and mean separation at 5% level was done using Duncan's Multiple Range Test and least significant difference (LSD).

Data collection and statistical analysis

Every 48 h, germinated seeds were counted and then discarded. A seed was regarded germinated once the tip of the

radical had grown free of the seed coat (Wiese & Binning, 1987; Auld et al., 1988). The subsequent germination parameters were noted:

(a) Germination percentage (GP) = (number of germinated seeds/number of tested seeds) $\times 100$.

(b) Germination rate index (GRI) = $[(G1/1) + (G2/2) + (Gx/X)]$

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

(c) Corrected germination rate index (CGRI) = $(\text{GRI}/\text{FGP}) \times 100$

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 20) software. The mean separations were done using Duncan's multiple range tests (Duncan, 1955) and significance was determined at $p \leq 0.05$.

Results and Discussion

Chemical Scarification

Effect of sodium hydroxide treatment on germination

The results in (Table 1) shows the treatments effect with sodium hydroxide, the germination percentages (GP) of date palm seeds that pre-germinated in soil or *in vitro* is increased till 30 min time course with 76.7 and 83.3 %, respectively, afterwards started to decrease. The number of days lapsed to reach 50% of GP (GT_{50}) of 7.75 and 7.48 days, the germination speed GRI and CGRI of seeds were no significant different in the treatments of sodium hydroxide for 20 or 30 min of pre-germinated seeds in soil or *in vitro* germinated.

The results in Table 1 show that the treatment of seeds with NaOH for 30 min in *in vitro* culture reached 83.30%

Table 1. Effect of sodium hydroxide (1N NaOH) treatment duration on germination percentage (GP), time to 50% of final germination (GT_{50}), CGRI and GRI for seeds that germinated in soil and *in vitro* culture

Treatment medium	GP		GT_{50} (days)		CGRI		GRI	
	in soil	<i>in vitro</i>	in soil	<i>in vitro</i>	in soil	<i>in vitro</i>	in soil	<i>in vitro</i>
0 (control)	70.0 a	80.0a	14.5a	12.0a	9.107c	11.118c	6.375c	8.89c
10	56.67 b	66.67b	13.44a	11.17a	8.616c	10.304d	4.883d	6.87d
20	63.33 b	70.0 b	10.6b	8.8c	11.50b	16.091a	7.283b	11.26b
30	76.70 a	83.30 a	7.75c	7.48c	15.42a	16.994a	11.82a	14.11a
40	30.00 c	43.33 c	8.5bc	7.73c	15.37a	13.133b	4.611d	5.69de
50	16.70 d	26.70 d	9.5bc	7.20cd	11.02b	17.562a	2.942e	4.69e

Means in the same column with the same letter(s) are not significantly different at $P = 0.05$, according to Duncan's Multiple Range Test

survival, 7.48 GT₅₀, 16.99 CGRI and 14.105 GRI. This is in agreement with the research on freshly-collected date palm seeds by Moussa et al. (1998). The seed of many species (*palmease*) showed positive response to several pretreatment such as stratification, scarification, and exposure to some chemical materials (gibberellic acid) (Arteca, 2013; Frett, 1987; Hartmann et al., 1990; Liopa-Tsakalidi & Barouchas, 2011; Schopmeyer, 1974). In some previous studies it has been revealed that scarification intensified the germination percent of palm seeds with water-impermeable hard endocarp (Marcus & Banks, 1999a; Murakami and Rauch, 1998; Nagao et al., 1980). These method are recommended only for those seeds that are very hard to germinate, as damage caused to the embryo during the process might be high (de Dios Holmquist & Popenoe, 1967; Meerow & Broschat, 1991; Nagao et al., 1980; Odetola, 1987).

Effect of potassium nitrate treatment on germination

The experiment showed that the treatment with potassium nitrate affect the viability of the seeds, as suggested by the germination percentage (Table 2). There was a significant increase in germination percentage with increasing the exposure treatment time of seeds that pre-germinated in soil or *in vitro*. When the exposure time reached 30 min the viability of the seeds was highest as showed by the high GP it was 76.67 and 80.0%, respectively, but significant decrease happened with other treatment (Table 2). The GP of the seeds showed reduction during high exposure time. Likewise, the experiment exhibited decreased values for GT₅₀, whereas those of treatment time increased notably by all treatments, in relationship with the control method. Seed germination rate increases with the decrease of GT₅₀ (Angus et al., 1981; Fulbright & Fulbright, 1990; Hsu et al., 1985; Kanemasu et al., 1975). On the other hand several researchers reported that plant growth and productivity is affected by many biotic and abiotic factors (Turk and Tawaha, 2001; Tawaha & Turk, 2002; Turk & Tawaha, 2002a; Turk & Tawaha, 2002b; Tawaha et al., 2003;

Turk et al., 2003; Musallam et al., 2004; Nikus et al., 2004; Al-Tawaha et al., 2005; Tawaha et al., 2006; Al-Rifaei et al., 2007; Tawaha & Al-Ghzawi, 2013; Al-Tawaha et al., 2017; Al-Tawaha et al., 2018a; Al-Tawaha et al., 2018b; Al-Ghzawi et al., 2019).

As compared with the control, control shown maximum GP then treated with potassium nitrate (1N KNO₃) for 30 min shown highest GP. The GT₅₀ values decreased, whereas those of treatment time increased significantly by all treatments, in comparison with the control as with many other plant species (Mayer & Poljakoff-Mayber, 1989). The reason for increase seed germination rate of date palm by chemical scarification was the increased seed coat permeability to water and/or gases. Hence, these results signified the connection of these two constraints in the slow seed germination of date palm. Additionally, other unidentified aspects, possibly chemical inhibitors (Ahmed, 1989a), could also be implicated in all experiments.

The nitrates have been widely used to overcome seed dormancy (Nadjafi et al., 2006). The best germination percentages were achieved in 1% for KNO₃ treatments of *Sabal*, in which maximum germination of 90% were attained (Dewir et al., 2011). It is 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 & Black, 1994). In Dewir et al. (2011) study for *Thrinax* and *Sabal* seeds find different trend when compared of KNO₃ and GA3 treatments.

Effect of sulfuric acid treatment on germination

The treatment with concentrated sulfuric acid (scarifying agent) (El-Sheikh, 1988) in comparison with the control affect the germination percentage during 10, 20, 30, 40 and 50 min. A reduction in the GP was observed when the treatment time was increased to 40 and 50 min as can be seen in Table 3. The maximum GT₅₀ were showed by the control. As treatment period was increased the two indices steadily decreased and increased, respectively (Table 3).

Table 2. Effect of potassium nitrate (1N KNO₃) treatment duration on germination percentage, time to 50% of final germination (GT₅₀) CGRI and GRI in seeds that germinated in soil and *in vitro* culture

Treatment medium	GP		GT ₅₀ (days)		CGRI		GRI	
	in soil	<i>in vitro</i>	in soil	<i>in vitro</i>	in soil	<i>in vitro</i>	in soil	<i>in vitro</i>
0 (control)	70.00 a	70.00 a	14.5a	11.83a	9.17d	10.92b	6.42b	7.64b
10	46.67 c	56.67 b	12.91b	10.89a	10.93cd	8.769c	5.11c	6.13b
20	63.33 b	70.00 a	10.38c	8.00b	11.68cd	17.425a	7.40b	12.12a
30	76.67 a	80.00 a	7.75e	3.70d	17.66a	18.767a	13.55a	14.40a
40	46.67 c	43.33 d	8.66d	6.96c	15.29ab	16.116a	7.15b	6.98b
50	20.00 d	13.30 c	9.47cd	7.475c	12.45c	16.68a	2.90d	2.79c

Means in the same column with the same letter(s) are not significantly different at P = 0.05, according to Duncan's Multiple Range Test

Table 3. Effect of concentrated sulfuric acid (H₂SO₄) treatment duration on germination percentage, time to 50% of final germination (GT₅₀) CGRI and GRI in seeds that germinated in soil and *in vitro* culture

Treatment medium	Germination %		GT ₅₀ (days)		CGRI		GRI	
	in soil	<i>in vitro</i>	in soil	<i>in vitro</i>	in soil	<i>in vitro</i>	in soil	<i>in vitro</i>
0 (control)	73.33 b	73.33bc	12.68a	12.03a	10.67d	11.59c	7.824c	8.495d
10	80.00b	86.87ab	12.36a	9.06b	8.043e	15.32b	6.435d	13.28b
20	93.00 a	96.67a	9.52b	5.82d	14.32ab	18.80a	13.36a	18.18a
30	90.00 a	93.33a	7.27c	5.12d	17.43a	18.79a	15.69a	17.53a
40	53.30 c	63.30c	8.19bc	5.29d	16.89a	18.65a	9.00b	11.8bc
50	26.70 d	36.70d	9.01b	7.46c	12.79cd	17.01ab	3.42e	6.24e

Means in the same column with the same letter(s) are not significantly different at P = 0.05, according to Duncan's Multiple Range Test

The GT₅₀ values demonstrated by the seeds treated for 20 and 30 min were not drastically different from each other, whereas they were significantly different from the values of those treated for 10 min and the control. Hence, treating the seeds for 30 min was amongst the treatments which caused in the maximum germination rates as well as among those that leads in the highest germination percentages. Thus, treating the seeds with concentrated sulfuric acid for 30 min might be used effectively as a simply accomplished practical means to enhance the seed germination rate of date palm. These pretreatments are not always reliable with cycads (Dehghan & Yuen, 1983). Few studies showed that the seeds of cycads germinated better after exposure to sulfuric acid, GA, darkness and mechanical pretreatments (Dehghan & Yuen, 1983; Frett, 1987).

The seed germination of the various hard-seeded species of palm are promoted by several pretreatments such as mechanical and chemical scarification (Dewir et al., 2011; Frett, 1987; Rouhi et al., 2010; Tigabu & Oden, 2001). The seed of many species (*palmease*) showed positive response to several pretreatment such as stratification, scarification, and exposure to some chemical materials (gibberellic acid) (Arteca, 2013; Frett, 1987; Hartmann et al., 1990; Liopa-Tsakalidi and Barouchas, 2011; Schopmeyer, 1974). In some previous

studies it has been revealed that scarification intensified the germination percent of palm seeds with water-impermeable hard endocarp (Marcus and Banks, 1999a; Murakami and Rauch, 1998; Nagao et al., 1980). These method are recommended only for those seeds that are very hard to germinate, as damage caused to the embryo during the process might be high (de Dios Holmquist & Popenoe, 1967; Meerow & Broschat, 1991; Nagao et al., 1980; Odetola, 1987).

Mechanical Scarification

Effect of mechanical treatment on germination

As compared to the untreated seeds there was a progressive response to mechanical scarification treatments by seed germination. It can be seen from data in Table 4 there is a prominent difference in GP between the different treatments. High germination percentage of 76.7% was observed when intact operculum with scarification was done. However, 66.7% germination was shown during the treatment with completely remove operculum and Partial remove operculum with scarification. Lowest GP of 56.7% was observed in scarification of seed partial: remove operculum but resulted in considerably same germination percentage as compared to untreated seed (Intact operculum) which failed to germinate.

Table 4. Effect of mechanical treatment in germination percentage, time to 50% of final germination (GT₅₀) CGRI and GRI in seeds that germinated in soil and *in vitro* culture

Treatment	GP		GT ₅₀		CGRI		GRI	
	in soil	<i>in vitro</i>	in soil	<i>in vitro</i>	in soil	<i>in vitro</i>	in soil	<i>in vitro</i>
A (control)	73.30a	76.76ab	12.68a	11.47a	11.53b	11.661c	8.45c	8.94d
B	56.67cb	66.67bc	9.55b	7.71b	12.20b	17.30a	6.92d	11.54bc
C	66.67ab	76.67ab	7.49c	7.08b	18.085a	17.42a	12.06a	13.36ab
D	76.67a	86.67a	6.93c	6.20bc	17.42a	18.17a	13.36a	15.75a
E	66.67ab	76.67ab	7.49c	6.86b	17.14a	17.42a	11.43ab	13.36ab
F	63.33bc	70.00b	9.65b	8.00b	16.27a	16.70ab	10.230b	11.69bc

Means in the same column with the same letter(s) are not significantly different at P = 0.05, according to Duncan's Multiple Range Test

A: Intact operculum (control), B: Partial removed operculum, C: completely removed operculum, D: Intact operculum with scarification, E: Partial removed operculum with scarification, and F: Completely removed operculum with scarification

The main factors that affected the number of days consumed for seed germination is mechanical scarification as indicated by the various analysis. It can be seen in Table 4 that there is a notable decrease from 12.68 in average number of germination GT_{50} in the seeds intact operculum to 6.93 in the seeds scarified at intact operculum with scarification. However seed coat scarification at completely remove operculum was 9.55 and at partial remove operculum was 7.49. Seeds belonging to many species showed that mechanical scarification is easy and efficient method. Scarified seeds are more susceptible to damage from pathogenic organisms as compared to non-scarified seeds. Prior to sowing certain aspects should be considered. Firstly, scarification must not continue to the point at which the seeds are damaged. Secondly, the seed coats must be dull and not so deeply pitted or cracked for avoiding the exposure of the inner part of the seed. For optimum time determination, the seeds might be drenched to note swelling, or the seed coats may be studied with a hand lens (Bonner et al., 1974; Schopmeyer, 1974).

Conclusion

This study presents a laboratory attempt to explore the effect of scarification treatment of Date Palm Seeds (*Phoenix dactylifera* L.). It showed that scarification treatments are helpful in increasing the germination rate and also the early growth of the seedlings. Hard endocarp surrounding the Date Palm Seeds (*Phoenix dactylifera* L.) were more efficiently removed by sulphuric acid treatments rather than mechanical scarification. Though, the results exhibited that date palm seed showed exogenous dormancy which is completely imposed by the hard seed coat. The outcomes resulting from this experiment suggest that the different treatments used had substantial effect on germination of date palm. But, this reaction was not uniform with all treatments. The difference in the response may be owed to variant in the effect of sulfuric acid, potassium nitrate, sodium hydroxide and mechanical scarification on removing and/or cracking of the test surface and consequently raising the permeability of the seeds to water. Use of tissue culture or sulfuric acid to overcome date palm seed dormancy is vital particularly for local farmers, to lessen the production cost using acid where it is sufficiently not available but it is not for the large-scale date palm cultivators. This procedure may offer an establishment for advancement of generalizable seed germination method for other plant species as this method is simple, quick, economical, effective and repeatable. Moreover can be done on on year-round basis. Though, for commercial application further research is necessary to optimize total germination percentages and to reduce the number of days needed for

germination. Further research is being carried out at genetic and molecular levels in our laboratory

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