Effect of Fruit Developmental Stage, Seed Scarification and Operculum Removal on Seed Germination of Date Palm

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Abstract. The germination responses of date palm (*Phoenix dactylifera* L.) cv Ruzeiz seeds, extracted from fruits at the mature green (Kimri), yellow (Khalal), early ripe (Rutab) and ripe dry (Tamar) stages, to seed scarification and/or operculum removal were investigated. The percentages of germination were above 90% and below 75% of the seeds with and without opercula, respectively. Higher germination rates were exhibited by the Kimri and Khalal seeds, irrespective of seed treatments. The seed scarification significantly improved germination rates of all seeds. The highest levels of germination rates were exhibited by the operculum removed at Kimri and Khalal stages. They germinated within the first two days, whereas none of the other seeds germinated before the third day, of seed incubation. Thus, the comparatively low germination rate of the Kimri and Khalal intact seeds could be attributed to the impermeability of seed coat to water and/or gases and the mechanical resistance of the operculum to the embryo elongation, in addition to certain chemical inhibitor(s) for that of the Rutab and Tamar seeds. A significantly lower *in vitro* growth rate was attained by the Tamar-isolated embryoes, in comparison with the Khalal. This implies that the site of the chemical inhibitor(s) involved in the low germination rate of the Tamar seeds was probably the embryo itself rather than the surrounding tissues. Also, the embryo culture of the Kimri and Khalal stages may be preferred to that of the Rutab and Tamar stages when the time factor is critical, since it is easier to isolate the embryoes and they are faster in growth.

Introduction

Occasionally, the date palm (*Phoenix dactylifera* L.) seedlings have been utilized in certain short term studies [1; 2]. As with many other palm species belonging to the palm family, Arecaceae [3, p. 20; 4, p. 482; 5], the germination rate of date palm is comparatively low [6; 7]. Hence, the improvement of seed germination rate of this species might be desirable in such investigations. A remarkable improvement of the germination rate of date palm seeds extracted from fully ripe fruits had been achieved by seed scarification [8]. However, in these studies the germination rate was still lower than the normal levels, even at the supra-optimal temperature. This indicates that a further

improvement of seed germination rate of date palm would be possible; should the responsible constraints are eliminated or should they have not been present. The onset of both embryo and seed coat-imposed dormancy in many plant species such as *Acer pseudoplatanus* L, *Sida spinosa* L. [9, pp. 78, 104], *Medicago lupulina* L. [10], *Vicia faba* L. and *Trifolium alexandrinium* L. [11, pp. 81-110] may be restricted to the final stages of, or it may take place earlier, but the proportion of the dormant seeds would increase with, seed maturity. As yet, it is not known whether the delay in the seed germination of date palm is the case here, as well. The present study was conducted to investigate the separate and the interactive effects of the fruit developmental stage, the seed scarification and the removal of the opercula, on the germination of date palm seeds.

Materials and Methods

Date palm fruits were hand-picked at the fully developed green (Kimri), yellow glossy (Khalal), early ripe (Rutab) and fully ripe fairly dry (Tamar) stages [12] from cv Ruzeiz trees grown at the College of Agriculture and Veterinary Medicine, King Saud University, Research Station at Al-Qassim, Saudi Arabia. Seeds were extracted, washed with tap water, wiped with paper towels and stored separately in polyethylene bags at 3-5° C. Eighty seeds of each stage were surface sterilized with 2.5% sodium hypochlorite solution for 20 minutes [7]. Then, they were washed with, and soaked in, sterilized distilled water at 30° C for 6 h. The operculum (The umbiliform structure overlying the embryo in the micropylar region [13, p. 479]) of each of 40 of these seeds was removed under a dissecting microscope using a scalpel fitted with a surgical blade. Care was taken not to damage the embryoes. Twenty of both the operculum-removed seeds and the seeds with opercula were scarified by cutting the tips of each seed with a pruning clipper at about half the distance between the operculum and the tip. The remaining twenty intact seeds were used as a control.

Based on the results of the above experiment, another experiment was designed to find out whether the mechanical resistance exerted by the operculum on the embryo was high enough to delay its protrusion. Sixty seeds of each stage were surface sterilized, washed, soaked and scarified as described previously. The central portion of the operculum of 20 seeds of each stage was excised under a dissecting microscope by moving a surgical blade, fitted on scalpel, horizontally along the dorsal surface. This would open a small passage for the influx of water and gases, while the ring-shaped remaining portion of the operculum would exert the mechanical resistance that the intact operculum might have had. The opercula of other 20 seeds of each stage were removed completely, as in the first experiment. The remaining 20 seeds were used as a control.

The seeds were evenly distributed in a 14x2.5 cm plastic Petri dish, half-filled with washed, air-dried and sterilized coarse sand. With the dorsal side oriented upward, each seed was placed in a small hole made in the soil surface which had been wetted with a 2% (w/v) Vitavax (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) fungicide

solution. All dishes were put in plastic bags to prevent the evaporative water loss and then arranged in a completely randomized design inside an oven set at 30±1° C. In both experiments, each treatment was replicated four times. Germinated seeds were counted and discarded daily, until no further germination was observed for five successive days. A seed was considered germinated when the proximal end of the cotyledonary sheath had protruded beyond the seed coat. The germination percentage and the time to reach 50% of the final germination (GT_{50}) were determined. The germination rate index (GRI) was calculated as the summation of the germination percentage for each day divided by the total number of days for germination [14]. The corrected germination rate index (CGRI) was obtained by dividing GRI by the final germination percentage and then multiplying by 100 [15].

The germination percentages were arcsin, whereas the GT_{50} values were square root $(X + 0.5)^{1/2}$, transformed, prior to statistical analysis [16, pp 304-308]. However, the data presented in the text are untransformed means.

In vitro experiment was conducted to compare the growth rate of the Khalal-isolated embryoes isolated with Tamar-isolated embryoes. Some seeds of Khalal and Tamar stages were extracted. The Tamar stage seeds were soaked in sterile distilled water for 24 h to facilitate the embryoes isolation. The seeds of both stages were disinfected by 10% Clorox (5.25% sodium hypochlorite) plus 2 drops of Tween 20/100 ml for 20 min, and then rinsed three times with sterile distilled water. Using a pruning clipper, each seed was cut at the operculum region. Then, the embryoes were isolated by a surgical blade and cultured on MS medium [17] supplemented with 30 g sucrose and 7 g agar per liter (Micro Agar, DUCHEF Biochemicals, The Netherlands). The pH of the medium was adjusted to 5.7 before the addition of the agar and autoclaving. All cultures were maintained under 16 h photoperiod (cool white flourescent light) with irradiance of 2500-3000 lux at 27±2° C. The lengths of embryoes were measured every other day, using a dissecting microscope fitted with a calibrated eyepiece micrometer.

Analysis of variance was carried out using WINKS statistical data analysis program (TexaSoft, Cedar Hill, Texas, USA).Treatment means were statistically compared by using Newman-Keuls test.

Results

Above 90% of the intact and the scarified seeds, of all stages, with opercula germinated, whereas the operculum-removed seeds showed significantly lower germination percentages ($\leq73\%$) (Table 1); the Rutab and the Tamar seeds were more affected. The embryoes of operculum-removed seeds which failed to germinate were fungal infected within the first 24 h of seed incubation, whereas, none of the embryoes of the other similarly treated seeds were infected.

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The corrected germination rate index (CGRI) values exhibited by the Kimri and the Khalal seeds were significantly higher than those exhibited by other stages for most of the seed treatments (Table 2). On the other hand, there were no statistically significant differences between the Kimri and the Khalal, and between the Rutab and the Tamar, seeds. Seed scarification and operculum removal increased the CGRI values significantly, in comparison with the control. The highest values were attained by the operculum-removed, and/or operculum-removed scarified, seeds (Table 2).

Table 1. The effect of fruit developmental stage, seed scarification and the removal of the opercula on the germination percentage of date palm seeds

| Seed condition | Fruit developmental stage | | | | |
	Kimri	Khalal	Rutab	Tamar	Average
Intact seeds					
With opercula	91.25^1Aa^2	95.00Aa	93.75Aa	93.75Aa	93.44a
Without opercula	71.25Ab	73.75Ab	66.25Ab	56.25Bb	66.88b
Scarified seeds					
With opercula	92.50Aa	93.75Aa	95.00Aa	95.00Aa	94.06a
Without opercula	68.75Ab	72.50Ab	61.25Ab	60.00Ab	65.63b
Average	80.94A	83.75A	79.06A	76.25A	

[1]Percentage germination data were arsin transformed for analysis.
[2]Mean separation by Newman-Keuls multiple comparison test; 5% level. Upper case letters for rows and lower case letters for the columns.

Table 2. The effect of the fruit developmental stage, seed scarification and the removal of the opercula on the corrected germination rate index (CGRI) (day^{-1}) of date palm seeds

| Seed condition | Fruit developmental stage | | | | |
	Kimri	Khalal	Rutab	Tamar	Average
Intact seeds					
With opercula	$31.53Ac^1$	35.48Ac	22.83Bd	20.90Bd	27.69c
Without opercula	73.52Aa	75.53Aa	53.65Bb	51.93Bb	63.66a
Scarified seeds					
With opercula	49.56Ab	46.09ABb	41.17BCc	38.90Cc	43.93b
Without opercula	76.94Aa	71.99Aa	60.68Ba	58.41Ba	67.01a
Average	57.91A	57.28A	44.59B	42.53B	

[1]Mean separation by Newman-Keuls multiple comparison test; 5% level. Upper case letters for rows and lower case letters for the columns.

The time to reach 50% of the final germination (GT_{50}) values exhibited by the Kimri and Khalal seeds were lower than those exhibited by the other seeds (Table 3). As with CGRI, the Kimri and the Khalal seeds attained almost similar GT_{50} values; and likewise were the Rutab and the Tamar seeds. Both seed scarification and operculum removal reduced the GT_{50} values significantly (Table 3).

Table 3. The effect of fruit developmental stage, seed scarification and the removal of the opercula on the time to 50% of final germination percentage (GT_{50}) (days) of date palm seeds

Seed condition	Fruit developmental stage				
	Kimri	Khalal	Rutab	Tamar	Average
Intact seeds					
With opercula	8.25[1]Aa[2]	8.50Aa	11.25Ba	11.00Ba	9.75a
Without opercula	1.50Bc	1.75Bc	5.75Ab	6.00Ab	3.75c
Scarified seeds					
With opercula	5.75ABb	5.50Bb	6.50ABb	7.00Ab	6.19b
Without opercula	1.25Bc	1.00Bc	4.00Ac	4.25Ac	2.81c
Average	4.19B	4.25B	7.06A	7.00A	

[1]Days to 50% germination data were arcsin transformed, $(x + 0.5)^{1/2}$, for analysis.
[2]Mean separation by Newman-Keuls multiple comparison test; 5% level. Upper case letters for rows and lower case letters for the columns.

Excluding the control, the highest GT_{50} values were exhibited by the scarified seeds, irrespective of the stages. The lowest GT_{50} values for the Rutab and the Tamar, were exhibited by the operculum-removed scarified, seeds, in addition to the operculum-removed, for the Kimri and the Khalal, seeds.

The interactive effects of the fruit developmental stage at which the seeds were extracted and the seed treatment, revealed that the highest and the lowest CGRI and GT_{50} values, respectively, were exhibited by the operculum-removed intact and scarified Kimri and Khalal seeds (Tables 2 and 3). The next highest and the next lowest GT_{50} and CGRI values, respectively, were attained by the operculum-removed scarified Rutab and Tamar seeds. All the viable operculum-removed scarified Kimri and Khalal seeds, with intact embryoes, germinated within the first 2 days, whereas none of the Rutab and the Tamar seeds germinated before the third day, of seed incubation.

Comparing the GT_{50} and CGRI values exhibited by the scarified seeds from which only the central portions of the opercula were excised with those exhibited by the scarified seeds, shows that the differences were negligible, irrespective of the fruit stage. In contrast, the differences between the former and the operculum-removed scarified seeds were statistically significant (Table 4).

Table 4. The effect of partial and complete removal of the operculum on the time to reach 50% of the final germination (GT_{50}) (days) and the corrected germination rate index (CGRI) (day[-1]) of the scarified date palm cv Ruzeiz seeds at various fruit developmental stages

Fruit developmental stage germination parameter	Kimri		Khalal		Rutab		Tamar	
	GT_{50}[1]	CGRI	GT_{50}	CGRI	GT_{50}	CGRI	GT_{50}	CGRI
Intact operculum	8.75a[2]	36.29b	8.00a	32.98b	11.50a	23.68c	11.25a	19.87c
Partially removed operculum	7.75a	39.76b	7.50a	35.94b	8.50b	38.14b	7.75b	45.76b
Completely removed operculum	1.25b	78.92a	1.50b	80.04a	5.50c	59.27a	5.25c	63.32a

[1]GT50 values were square-root transformed, $(x + 0.5)^{1/2}$, for analysis.
[2]Mean separation by Newman-Keuls multiple comparison test; 5% level.

Figure1 shows the *in vitro* growth patterns of the embryos extracted from the Khalal and the Tamar seeds. The embryos of both types of seeds were about 2 mm in length when they were extracted. The Khalal embryos started to elongate at or before the second day, while the Tamar embryos did so after the forth day, of embryo culture. The Khalal embryos were longer than the Tamar embryos, throughout the experimental period. The differences were statistically significant.

Discussion

The corrected germination rate index (CGRI) and the time to reach 50% of the final germination (GT_{50}) have been widely used to compare the relative rate of germination [18; 19] and to evaluate the seed germination rate in meaningful biological units [20; 21], respectively. Evaluated by these two indices, higher seed germination rates were exhibited by the Kimri and the Khalal seeds (Tables 2 and 3). This is probably due to the formation of certain germination delaying factors during the later stages of date palm seeds development, as reported with some other plant species [9, 78, 104].

Scarifying the seeds by cutting both ends of each seed enhanced the germination rate of the Kimri, Khalal, Rutab and the Tamar seeds, remarkably (Tables 2 and 3). The improvement of the germination rate of the Tamar seed by mechanical seed scarification was also reported by Al-Salih [8]. Thus, it seems that the seed coat impermeability to water and/or gases is implicated in the delay of the seed germination of date palm. This phenomenon is also responsible for the seed dormancy of a multitude of both horticultural and field crops [22, pp. 53-57].

A further improvement of the germination rate was brought about by the removal of the opercula, by itself or in addition to seed scarification (Tables 2 and 3); which also reduced the seed germination percentage, probably due to the failure of the embryoes, which had been damaged, to elongate. Beside exposing the embryo, the removal of the operculum was a type of mechanical seed scarification. Therefore, the difference in the germination rate between these, and the scarified, seeds could be attributed to the influx of water and/or gases into the seeds through the opening made by the removal of the opercula and/or to the alleviation of the mechanical resistance, to the embryo elongation, which might have been exerted by the operculum.

The lack of statistically significant difference between the germination rate exhibited by the scarified seeds and that exhibited by the scarified seeds from which the central portions of the opercula were excised (Table 4) and thus opened small passages for the influx of water and gases, while the ring shaped remaining portions would exert almost the same mechanical resistance that the intact operculum might have had, indicated that the influx of water and gases through the opening made by the removal of the operculum had no significant effect. In contrast, the significantly lower seed germination rate exhibited by the later, compared with the operculum-removed scarified, seeds (Table 4), indicates that this difference was due to the removal of the opercula. The combination of _

these information revealed that the lower germination rate exhibited by the scarified seeds in comparison with the operculum-removed scarified seeds could be attributed to the mechanical resistance of the opercula to the embryo elongation. Hence, it is apparent that the opercula exerted enough mechanical resistance, to the embryo elongation, to be implicated in the delay of the seed germination of date palm. Reduction of the seed germination rate resulting from the mechanical resistance of the embryo-surrounding tissue was also reported with lettuce, *Lactuca sativa* L.[9, pp. 78, 104].

Apparently, the removal of the opercula of the intact and the scarified Kimri and Khalal seeds increased their germination rate to attain the normal level. Thus, evidently these treatments had eliminated all the constraints responsible for the delay of the germination of these seeds. On the other had, the germination rates exhibited by the similarly treated Rutab and Tamar seeds were still far less than the normal level. This indicates that certain germination constraints other than those eliminated by the removal of the opercula were still active in the scarified Rutab and Tamar seeds , most likely, these were chemical inhibitors. Working with the Tamar seeds of the same cultivar, Ahmed [6] reported the presence of such chemicals and attributed the delay of germination to their action. It seems that these chemicals were formed at, or increased in concentration with, the commencement of fruit ripening. The site of these inhibitors was probably the embryo itself, rather than the surrounding tissues, as depicted by the substantially lower *in vitro* growth rate of Tamar, in comparison with the Khalal, embryoes (Fig.1).
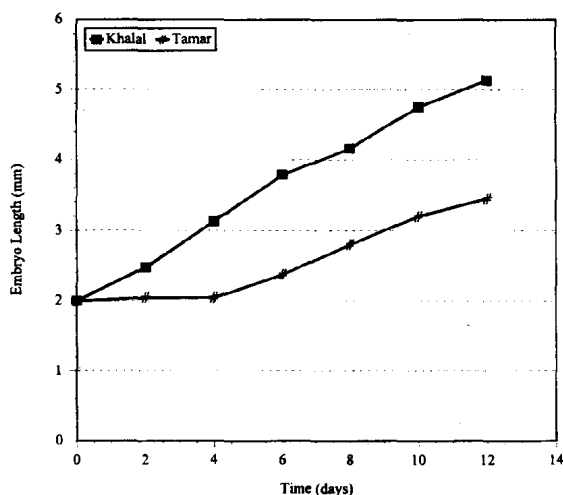

Fig. 1. *In vitro* elongation of the embryoes isolated from the Khalal
and the Tamar date palm seeds>.

Conclusion

A higher germination rate was exhibited by the Kimri and Khalal, in comparison with the Rutab and the Tamar, seeds. The seed scarification significantly improved the germination rate of the seeds of all stages. A further improvement of this parameter was brought about by the removal of the opercula for the Kimri and the Khalal, or by the removal of the opercula combined with seed scarification for the Rutab and the Tamar seeds

The delay of the germination of the date palm seeds could be attributed to the impermeability of seed coat to water and/or gases and the mechanical resistance of the operculum to the embryo elongation, for the Kimri and the Khalal, in addition to certain chemical inhibitors for the Rutab and the Tamar, seeds.

A comparatively normal seed germination rate was exhibited by the Kimri and the Khalal seeds from which the opercula had been removed. At 30°C, these seeds attained the maximum cumulative percent germination within the first two days of seed incubation. However, the manual removal of the opercula is time consuming, and may reduce the percentage germination, due to the damage of the embryoes. Therefore, a search for an easily-performed method to remove the opercula, without damaging the embryoes, is warranted.

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تأثير مراحل نمو الثمار وخدش البذور وإزالة غطاء الجنين علي معدل إنبات بذور نخيل البلح

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ملخص البحث. استهدف هذا البحث دراسة تأثير مراحل نمو الثمار، وخدش البذور، عن طريق قطع طـــرف كــل بذرة، وإزالة القرص الذي يغطي الجنين، علي معدل إنبات بذور نخيل البلح الصنف رزيز والتي تم الحصول عليها من ثمار في أربع مراحل للنمو وهي الكمري (الثمار الخضراء مكتملة النمو) والخلال (الثمار الصفراء اللامعة) والرطب (بداية النضج) والتمر (ما بعد اكتمال النضج). وقد أوضحت النتائج أن نسبة إنبات البذور غير المعاملة والبـــذور المخدوشة بلغت أكثر من ٩٠٪، بينما بلغت أقل من ٧٥٪ للبذور الأخرى. وقد أعطت بذور الكمــري والخـلال معدل إنبات أعلى من بذور الرطب والتمر، بينما أدي إزالة غطاء الجنين فقط أو بالإضافة إلي خـــدش البـــذور إلي معدل إنبات أعلى، يلي ذلك خدش البذور، بالمقارنة مع البذور غير المعاملة.

أعطت ثمار مرحلتي بذور الكمري والخلال، المخدوشة وغير المخدوشة، التي أزيلت أغطية أجنتها، أعلى معدل إنبات حيث نبتت هذه البذور خلال يومين من زراعتها، بينما لم تبتدئ البذور الأخرى في الإنبات إلا بعد اليـــوم الثالث من الزراعة. أيضاً أعطت الأجنة المفصولة من بذور الخلال التي زرعت علي بيئة مورشيقي واسكوج معـــدل نمو أعلى من الأجنة المفصولة من بذور التمر. من ثم فإن تأخر إنبات بذور مرحلتي الكمري والخلال قد يعـــزى إلى عدم نفاذية البذور للماء والهواء ومقاومة غطاء الجنين، بالإضافة إلى احتمال تأثير بعض المثبطات الكيميائية بالنسبة لبذور مرحلتي الرطب والتمر.