Effects of some combined treatments on breaking the seed dormancy and enhancing the germination rate from different Morrocan varieties of date palm *Phoenix dactylifera* L

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Abstract

An accelerating worldwide trend toward planting elite cultivars is leading towards two types of date palm (*Phoenix dactylifera L*) propagation: offshoot propagation or a tissue culture propagation. However, the date palm diversity is nowadays facing crucial problems. The alternative for both these methods is seed propagation. Seed dormancy in date palm has continued to be challenging especially when cultivating through seeds. This study evaluated the efficiency of the operculum removal treatment combined with temperature on the seed dormancy for four different Moroccan cultivars (Najda, Boufegousse, Aziza, and Assian). A germination test was carried out using a completely randomized design. Seeds from the four cultivars were subjected to the pre-germination treatment and germination rate and speed index were measured. Results showed that the treatment significantly ($p<0.05$) affected the germination rate and percentage of all of the four cultivars of date palm. Germination rates were above 90% and below 20% in the treated and control seeds, respectively. Higher germination rates were found in Najda and Assian seedlings which also had the highest levels of the germination speed index. The study revealed that seed dormancy in date palm can best be overcome by a combined treatment of operculum removal and temperature.

Keywords: *Phoenix dactylifera L,* cultivar, operculum, germination percentage, speed index

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Introduction

In many arid areas around the world, date palm is one of the main horticultural crops and an important source of food for many populations. This tree is not only a providential tree for the Saharan populations but also a life symbol in hot desert areas. Geographical distribution between

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24° and 34°N latitude is considered as the main biodiversity center of date palm distribution (Abul-Soad et al., 2017). There are about 5000 date palm cultivars besides countless varietal strains throughout the world, but every date-producing country has only a few top commercial cultivars being cultivated and exported all over the world. The germination and plantation of date palm seed in 6000 years ago was the first step to reach the full domestication of this plant. Early on, farmers recognized that date palms were dioecious: female plants produced fruit while male plants produced only pollen. Seed propagated date palms yield approximately equal numbers of males and females. At some point, a farmer discovered that it was possible to separate basal offshoots from a mother's palm and transplant them to another location (Johnson, 2011). However, this practice has a negative consequence as well, for over time it has reduced the diversity of the general date genetic pool. Without sexual reproduction, there are no opportunities for new genotypic combinations to occur (Jaradat, 2011). The date palm diversity is nowadays facing crucial problems such as Bayoud disease and red palm weevil (RPW) besides water shortage, urban settlement and disruption, rapid soil, and genetic erosion (Sedra, 2011a; Abul-Soad, 2011). Despite all these problems, little is known about Moroccan date palm genetic diversity and resistance of different cultivars to Bayoud. In general, varieties presenting a high rate of genetic diversity are resistant to diseases et al., 2012).

Date fruits are rich in protein, vitamins, and mineral salts. That is why they represent an essential element of diet for the desert population (El-Juhany, 2010). World date production was about 7.6 million tons in 2014 (FAOSTAT, 2014). Due to the high demand for this product, the heterogeneity resulting from seed plantation is a limiting factor for this technique, as well as the extreme needs of the seed germination conditions, mainly an adequate water supply, a suitable temperature, and the normal composition of the atmosphere. Slow, irregular, and infective germination process for most species can make it a bit challenging for many farmers. Most date palms take up until 30 days or more to germinate, with an average germination rate of less than 50% (Berton et al., 2013). Improvement of germination rate would be very useful as seedlings are used also as a source of explants for in vitro tissue culture as well as in vivo manipulations (Warrag and Warrag, 2007). The current research can be one of the means to improve the germination capacity of the date palm seeds, as a first step towards encouraging more research for maintaining the biodiversity of this species.

The highlight of this study was to improve the seed germination rate of four different local cultivars by operculum removal combined with a suitable incubation temperature to identify the factors responsible for high germination and the best variety that respond best to the germination treatment. The four cultivars were chosen based on their special characters: (i) “Najda” cultivar is a Moroccan date palm cultivar (*Phoenix dactylifera L.*) resulting from the National Institute of Agronomical Research (INRA-Morocco) selection programs, and characterized by *Fusarium oxysporum f.sp.Albedinis* (Bayoud) (Sedra 2011b), (ii) “Aziza Bouzid” and “Boufeggous Gharas” are the best cultivars for Figuig oasis located in the South-East region near the Algerian borders and are considered as high-quality date besides “Mejhoul” and “Bouskri” cultivar (Hasanaoui et al., 2010), (iii) “Assian” is one of the most produced and consumed cultivars in Morocco due to its low price and availability (Hasnaoui et al., 2012).

Materials and Methods

Seed collection and sample preparation

A total of 90 seeds per treatment of four cultivars of date palm were collected from three different areas in Morocco. Najda seeds were supplied by the National Institute of Agronomical Research experimental domain Zagora. Boufeggous seeds were purchased from Errachidia local date market. Aziza and Assian seeds were purchased from Figuig local date market in the same period of the year. After collection, the seeds were stored and dried in the shade for 2 weeks.

Seeds preparation and germination

The germination experiment was conducted in Marrakech National Institute of Agronomical

Research, Genetic Phytopathology Laboratory. Ninety seeds from each cultivar (Najda, Boufegousse, Aziza, and Assian) were cleaned of plant debris with tap water, then weighed on an analytical balance 0.001 g precision, and soaked in tap water for two days. Once hydrated, the upper tegument of the seeds was cleaned using a blade, keeping the embryo intact. Then the seeds were disinfected with 1% sodium hypochlorite for 2 minutes followed by a triple wash with sterile tap water.

The seeds previously prepared were put into germination on a thin layer of sterile sand lightly hydrated and covered with another thin layer. The control seeds without any treatment were also put simultaneously on a thin layer of sand. Both treatment and control samples were brought into incubation in an oven with a temperature of 32 ℃, sprayed every 48 hours with sterilized water for 15 days. The germinated seeds were counted and weighed daily for 20 days. Three treatment were combined in this study to speed up the seed germination:

T1: Soaking the seeds overnight in normal tap water at ambient temperature before they were removed and air-dried.

T2: Removal of the upper tegument of the seeds

T3: Incubation temperature combined with constant hydration

Notes concerning germination rate, days taken for initiation of germination, 50% germination, and final germination were recorded. It was ensured that no seeds were germinated in 10 days preceding the final germination date. The germination rate was determined by counting the number of seeds germinated each day from initiation of germination to final germination and this was expressed as an actual “Rate”, i.e. the number per day (Bewley and Black, 1994):

Germination Rate = total number of seeds germinated / number of days from initiation of germination to final germination (1)

Germination percentage

The number of germinated seeds in each treatment were recorded regularly. The first date of germination (FDG) and the last date of germination (LDG) were also recorded.

At the end of the germination period, the seed germination percentage (G %) was calculated as below:

$$G\ (\%) = (NSG/NTS) \times 100 \quad (2)$$

where NSG: number of germinated seeds and NTS: total number of seeds planted.

The germination speed index (GSI) was estimated according to the method proposed by Maguire (1962) using the expression simplified by Wang et al. (2004):

$$GSI = \sum (Gt/Tt) \quad (3)$$

where Gt is the number of germinated seeds on the day of counting and Tt is the day of counting.

Statistical Analysis

All measurements were done in triplicate. Data analysis was carried out by ANOVA using IBM SPSS STATISTICS 20. Differences between pairs of means were evaluated on the basis of 95% confidence intervals. The level of significance was $p \leq 0.05$.

Results

The data related to various germination parameters (initiation of germination, 50% germination, final germination, and germination rate) as influenced by difference in the removal of operculum combined with temperature and water soaking treatments are presented in Table 1. The treatments applied significantly increased the rate of seed germination in the four cultivars compared with the control. The number of days taken for initiation of germination, 50% germination, and final germination of seeds were significantly different ($p<0.05$) compared with the control seeds (Table1).

Effect of the operculum removal on the germination percentage

Table 1
Effect of the treatments applied on the number of days until initiation of germination, 50% germination, final germination, and germination % in date palm seeds compared with the control seeds

	Parameters	Najda	Boufgouss	Assian	Aziza
Control	Initiation of germination	16.66 ±1.53 Aa	17.00±1.00 Aa	15.67±2.08 Aa	17±1.00 Aa
	50% germination	19.33±2.08 Aa	20.00±0.00 Aa	18.33±3.21 Aa	Nd
	Final germination	23.00±1.00 Aa	25.00±1.00 Aa	22.00±2.00 Aa	Nd
	Germination Rate	2.60±0.68 Aa	1.29±0.07 Ab	2.13±0.39 Aab	Nd
Treatment	Initiation of germination	9.33±1.15 Ba	14.33±0.57 Bb	11.33±0.57 Bc	16.33±0.57Bd
	50% germination	12.00±1.00 Ba	14.66±0.57 Bb	14.33±0.57 Ab	17.33±0.57c
	Final germination	16.33±0.57 Ba	18.33±0.57 Bb	16.33±0.57 Ba	20±1.00b
	Germination Rate	4.29±0.94 Aa	4.43±0.60 Ba	4.65±0.43 Ba	5.48±1.77a

Different small letters within a row indicate differences between cultivars in the same treatment (p<0.05). Different capital letters within a column indicate differences between treatments for the same cultivar (p<0.05).

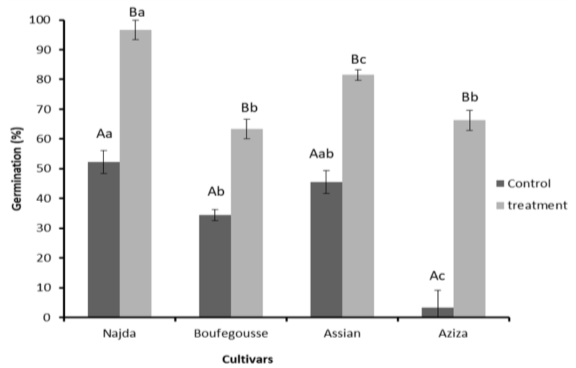

Fig. I. Effect of the treatment on the Germination %. Error bars represent the standard deviation (n=3). Bars with different capital letters indicate significant differences between treatments in the same cultivar (p≤0.05). Bars with different small letters indicate significant differences between cultivar in the same treatments (p≤0.05).

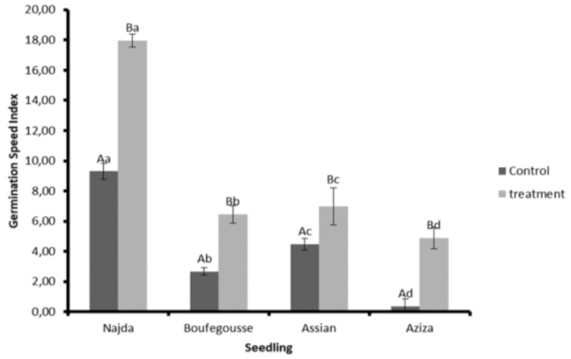

Fig. II. Effect of the treatment on the germination speed index; error bars represent the standard deviation (n=3). Bars with different capital letters indicate significant differences between treatments in the same cultivar (p≤0.05). Bars with different small letters indicate significant differences between cultivar in the same treatments (p≤0.05).

The effect of the treatment on the germination percentage of the four origins is represented in Fig. I. The mean germination percentage values for Najda, Boufeggous, Assian, and Aziza seedlings were 96.6±3.33, 63.3±1.92, 76.6±8.81, and 61.1±10.18%, respectively for the treatment and 53.3± 3.33, 34.4±1.92, 44.4±5.09, and 4.49±5.09%, respectively for the control.

The highest value of germination 96.66±3.33% was recorded for Najda variety and the lowest value 61,110±10.18% was recorded for Aziza variety for the treatment and according to ANOVA, there was a highly significant difference between and within groups p<0.05 (Fig. I).

Effect of the operculum removal on the Germination Speed Index of the tested seedlings

The results of the applied treatment effects on the germination speed index are presented in Fig. II. A significant effect was recorded (p<0.05) on the speed index germination for the four cultivars under study. SIG of treated seedlings reached a maximum of 17,944±0,433 for Najda variety compared with the control (9,295±0,299) while the minimum value was recorded in Aziza variety 4,869±0,718 compared with the control 0,351±0,501. According to the ANOVA test, a significant difference was found between and within groups (p<0.05). This treatment was able to improve the speed of the germination for the seeds; therefore, it helps with the time issue.

Kinetic of seeds germination as affected by operculum removal treatment during the germination period

The germination period was 15 days for the four cultivars, and the number of germinated seeds was recorded daily during the test period. The seeds maintained their germination capacity over the study period. Fig. III represents the evolution of the germination percentage for 15 days for the four treatments and for the control. The first germinated seed appeared in the fourth day in both control and treatment, for the Najda seeds and increased remarkably with time for the treated seeds compared with the control, and this was also noticed for the other three cultivars (Boufegousse, Assian, and Aziza). Najda variety showed the highest germination % reaching 97% in 15 days compared with the control reaching 50% in the same period (Fig. III).

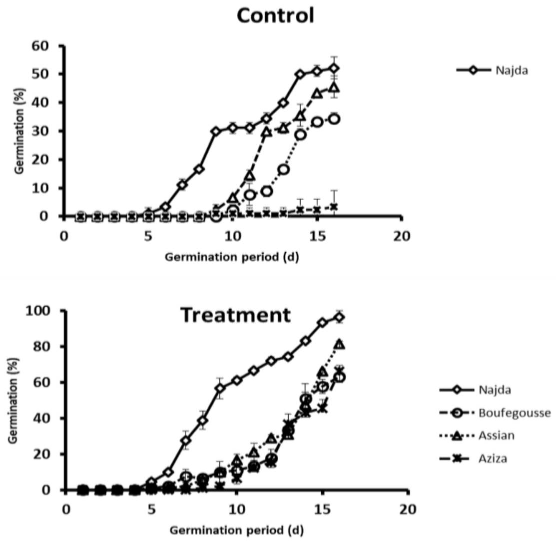

Fig. III. Germination percentage of control (A) and treated (B) seeds at 32 ℃ for 15 days; error bars represent the standard deviation (n = 30).

Discussion

In this study, we report the effects of some combined treatments (T1: Water: seeds were soaked overnight in normal tap water at ambient temperature, removed, and air-dried. T2: the removal of the upper tegument of the seeds. T3: the incubation temperature combined with constant hydration) on seed germination of four date palm cultivars. Information on seed germination requirements and effective dormancy-breaking procedures would benefit date palm seed propagation efforts. The germination percentage was used to evaluate seed germination. Based on this indices, higher seed germination rates were exhibited by the Najda and Assian seeds (Fig. I) and this can be explained with the formation of certain germination delaying factors during the later stages of date palm seeds development, as reported on some other plant species (Bewley and Black, 1994).

The removal of the opercula remarkably enhanced the Najda, Boufegousse, Assian, and even Aziza seedlings' germination speed index (Fig.II). The improvement of the germination rate of the other seedlings by mechanical seed scarification was also reported by Al Wasel and Warrag (1998). Thus, it seems that the seed coat of opercula can cause some impermeability to water and/or gases and it can be implicated in the delay of the seed germination of the date palm. This phenomenon is also responsible for the seed dormancy of a multitude of both horticultural and field crops (Mayer and Poljakoff-Mayber, 1989). The operculum removal can also be responsible for breaking the physiological dormancy by removing the resistance to radicle emergence by the seed or fruit coat (Bewley and Black, 1994).

A further improvement of the germination rate was brought about by the water soaking by itself or in addition to the temperature of the germination as also reported by Ganga and Radha (2017). Therefore, the difference in the germination rate between these combined treatments and the control can be attributed to the influx of water into the seeds through the opening made by the removal of the opercula, to the embryo elongation, which might have been exerted by the operculum.

Apparently, the removal of the opercula of the Najda and Assian seeds increased their germination rate to a very high level (mean germination percentages of 96.6±3.33% and 76.6±8.81%, respectively). Thus, these treatments had eliminated all the constraints responsible for the delay of the germination of these seeds. On the other hand, the germination rates exhibited by the similarly treated seeds of Boufegsous and Aziza seedlings were still less than 63.3±1.92 and 61.1±10.18%, respectively and this indicates that certain germination constraints other than those eliminated by the removal of the opercula were still active in the seeds and most likely, these were chemical inhibitors. Working with the seeds of a different cultivar, Warrag, and Warrag (2007) reported the presence of such chemicals and contributing to the delay of the germination process. It seems that these chemicals were formed at, or increased in concentration with the commencement of fruit ripening. These inhibitors could be located probably in the embryo itself, rather than the surrounding tissues.

Conclusion

In this study, the pre-germination treatments were most effective on the seed germination. A higher germination rate was exhibited by the Najda and Assian as compared with Boufeggous and the Aziza cultivars. Removing the opercula improved the germination rate in the seeds of all cultivars. A further improvement of this parameter was brought by the water soaking and the temperature of 32 ℃. The delay of the germination of the date palm seeds could be attributed to the impermeability of seed coat to water and the mechanical resistance of the operculum to the embryo elongation, in addition to certain chemical inhibitors.

A high seed germination rate was exhibited by the four s compared with the control. On the last day of the germination, these seeds reached the maximum cumulative percentage of germination. However, the manual removal of the opercula is time-consuming and may reduce the percentage of germination, due to the damage of the embryos. Therefore, applying a chemical treatment without damaging the embryos can be promising.

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