ORIGINAL ARTICLE

EFFECTS OF SOME COMBINED TREATMENTS ON BREAKING THE SEED DORMANCY AND ENHANCING THE GERMINATION RATE OF DIFFERENT MOROCCAN CULTIVARS OF DATE PALM

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ABSTRACT

Background: A growing global tendency towards the planting of elite cultivars leads to two types of Date Palm (Phoenix dactylifera L) propagation: offshoot propagation or tissue culture propagation. However, the date palm diversity is nowadays facing crucial problems. The alternative for both these methods is seed propagation. The propagation of date palm seeds is troublesome due to its long seed dormancy associated with poor seed germination. **Objectives**: 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). **Methods**: Thereby the germination test was carried out using a completely randomized design; the four cultivars seeds were selected and subsequently received the pre-germination treatment. In this survey, the evaluated traits were the germination rate, the germination speed index, and the Timson's index. **Results:** Results showed that the treatment significantly ($p < 0.05$) increased the germination rate and germination percentage for all the four cultivars of date palm compared to the non-treated plants. The germination rate was above 90% and below 20% for the treated seed and control, respectively. The "Najda" and "Assian" cultivars exhibited higher germination rates, in addition to the highest levels of the germination speed index. **Conclusions**: This study demonstrated that seed dormancy in date palm could best be resolved by an operculum removal treatment combined with temperature.

Keywords: *Phoenix dactylifera L, Dormancy; seed germination; operculum*

1. 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. With more than 5000 date palm cultivars and varietal strains all over the world, only few top cultivars are cultivated and exported worldwide [1]. 6000 years ago, the germination and plantation of date palm seed was the first step to reach the full domestication of this plant [2]. Date palms are known to be dioecious: female plants produce fruit while male plants produce only pollen. The seed propagated date palms produce about the same number of males and females [3]. In order to produce more good quality fruits, farmers separated basal offshoots from a mother's palm and transplant them to another location [3]. Nevertheless, the diversity of the general date genetic pool was reduced by using this practice over time. Without sexual reproduction, there are no chances for new genotypic combinations to occur [4]. The date palm diversity is nowadays facing crucial problems such as the epiphytic fungal disease caused by *Fusarium oxysporum f.sp. albedinis*, commonly known as "Bayoud" disease and red palm weevil (RPW), besides water shortage, urban settlement and disruption, rapid soil, and genetic erosion [1-5]. Despite all these problems, little is known about Moroccan date palm genetic diversity and the resistance of different cultivars to "Bayoud" disease. In general, cultivars presenting a high rate of genetic diversity are resistant to diseases [6].

Date fruits are rich in protein, vitamins, and mineral salts. That is why it represents an essential element of diet for the desert population [7]. World date production is about 7.6 million tons in 2014 [8]. 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 [9]. The slow, irregular and infective germination process for most species can be 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% [10]. Improvement of germination rate would be very useful as seedlings are used also as a source of explants for in vitro tissue culture manipulation as well as in other manipulations [11]. 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.

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Seed dormancy is described as a natural way of delaying the germination process and seeds to activate it when circumstances are normally suitable for germination and development of the seedlings. After that, the viable seeds that do not germinate are said to be dormant [12]. Although, seed dormancy is a plant protection mechanism to avoid germination under unsuitable conditions, it threatened the domestication and the cultivation of plants by seed propagation. In nature, there are different mechanisms to break the seed dormancy such as physical scraping of the seed coat to make it thinner so that water and gasses can spread through it, digestion of the seeds by animals, and the frosty conditions (humidity and heat). Some of the conventional methods used are also inspired by nature (scarification, treatment of seeds with chemicals like acids, soaking of seeds in water) [13].

The highlight of this study was to improve the seed germination rate of four different local cultivars by an operculum removal combined with a suitable incubation temperature, to identify the factors responsible for high germination and the best variety that responds well 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 its resistence to "Bayoud" [14]. (ii) "Aziza bouzid" and "Boufegousse gharas" are the best cultivars for Figuig oasis located in the South-east region near the Algerian borders and consider as high-quality date besides "Mejhoul" and "Bouskri" cultivars [15]. (iii) "Assian" is one of the most produced and consumed cultivars in Morocco due to its low price and disponibility [16].

2. MATERIALS AND METHODS

2.1. Seed collection and sample preparation

A total of ninety (90) seeds per treatment of four (4) 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. "Boufegousse" 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 two (2) weeks.

2.2. Seeds preparation and germination

The germination experiment was conducted in Marrakech National Institute of Agronomical Research, Genetic Phytopathology Laboratory.

Ninety (90) 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 with 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. Treatment samples were brought into incubation in an oven with a temperature of 32°C, sprayed every 48 hours with sterilized water for 15 days, while the control samples were put in ambient temperature 25°C. The germinated seeds were counted and weighed daily for 20 days. Three treatments were combined in this study to speed the seed germination:

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

Notes concerning germination rate, days that took for initiation of germination, 50% germination, and final germination, were recorded. It was ensured that no seed 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. number per day Eq.(1) [17].

$$Germination\ Rate = \frac{Total\ number\ of\ seeds\ germinated}{Number\ of\ days\ from\ initiation\ of\ germination\ to\ final\ germination} \quad (1)$$

2.3. Germination percentage

The number of germinated seeds in each treatment was recorded regularly. The first date of germination (FDG) and the last date of germination (LDG) were also recorded [18].
At the end of the germination test, the seed germination percentage (G %) was calculated as Eq.(2):

$$G\ (\%) = \left(\frac{NSG}{NTS}\right) \times 100 \quad (2)$$

Where, NSG: number of germinated seeds, 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) according to the Eq.(3) [19,20]:

$$GSI = \sum(^{Gt}/_{Tt}) \qquad (3)$$

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

The Timson's index was also used to estimate the speed of seed germination, the percentage of germination was recorded every 24h, at the end of 15 days, then all the results are summed [21].

$$Timson's\ index = \sum nG\ (\%) \qquad (4)$$

With n = cumulative daily germination percentage for each day of the test.

2.4. Statistical analysis

All measurements were done in triplicate. Data analysis was carried out by ANOVA two-way followed by tukey post-hoc test to assess differences between treatments and cultivars, using IBM SPSS STATISTICS 20. Differences between pairs of means were evaluated based on 95% confidence intervals. The level of significance was $p \leq 0.05$.

3. RESULTS

3.1. Germination parameters

The data related to various germination parameters (initiation of germination, 50% germination, final germination, germination rate and Timson's index) were presented in Table 1. Date palm seeds germinations were influenced by the operculum removal treatments combined with temperature and water soaking. The treatments applied significantly increased the rate of seed germination in the four cultivars compared to the control. The number of days taken for initiation of germination, 50% germination, and final germination of seeds were significantly lower $p<0.05$ for the treated seeds than the control seeds (Table 1).

Table 1: Effect of the treatments applied on the number of days until initiation of germination, 50% germination, final germination and germination % and Timson's index in date palm seed comparing with the control seeds.

	Parameters	Najda	Boufegousse	Assian	Aziza
Control	Initiation of germination	16.66 ±1.53 ^{Aa}	17±1.00 ^{Aa}	15.67±2.08 ^{Aa}	17±1.00 ^{Aa}
	50% germination	19.33±2.08 ^{Aa}	20±0.00 ^{Aa}	18.33±3.21 ^{Aa}	Nd
	Final germination	23±1.00 ^{Aa}	25±1.00 ^{Aa}	22±2.00 ^{Aa}	Nd
	Germination rate	2.60±0.68 ^{Aa}	1.29±0.07 ^{Ab}	2.13±0.39 ^{Aab}	Nd
	Timson's index	363.33±17.63 ^{Aa}	126.66±8.81^{Ab}	202.22±18.35 ^{Ac}	15.55±21.43^{Ad}
Treatment	Initiation of germination	9.33±1.15 ^{Ba}	14.33±0.57 ^{Bb}	11.33±0.57 ^{Bc}	16.33±0.57 ^{Bd}
	50% germination	12±1.00 ^{Ba}	14.66±0.57 ^{Bb}	14.33±0.57 ^{Ab}	17.33±0.57 ^{c}
	Final germination	16.33±0.57 ^{Ba}	18.33±0.57 ^{Bb}	16.33±0.57 ^{Ba}	20±1.00 ^{b}
	Germination Rate	4.29±0.94 ^{Aa}	4.43±0.60 ^{Ba}	4.65±0.43 ^{Ba}	5.48±1.77 ^{a}
	Timson's index	685.55±12.61^{Ba}	275.55±19.53 ^{Bb}	303.33±45.82 ^{Bc}	224.44±29.87 ^{Bd}

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 ($p<0.05$). Nd: Not determined.

3.2. Effect of the operculum removal on the Germination percentage (%)

The effect of the treatment on the germination percentage for the four seedlings was represented in figure 1. The germination percentage means for "Najda", "Boufegousse", "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, compared to 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 percentage 96.66±3.33 % was recorded for "Najda" cultivar and the lowest value 61,110±10.18 % was recorded for "Aziza" cultivar for the treatment experiment, and the statistical analysis showed that there was a highly significant difference between and within the groups $p < 0.05$ (Figure 1).

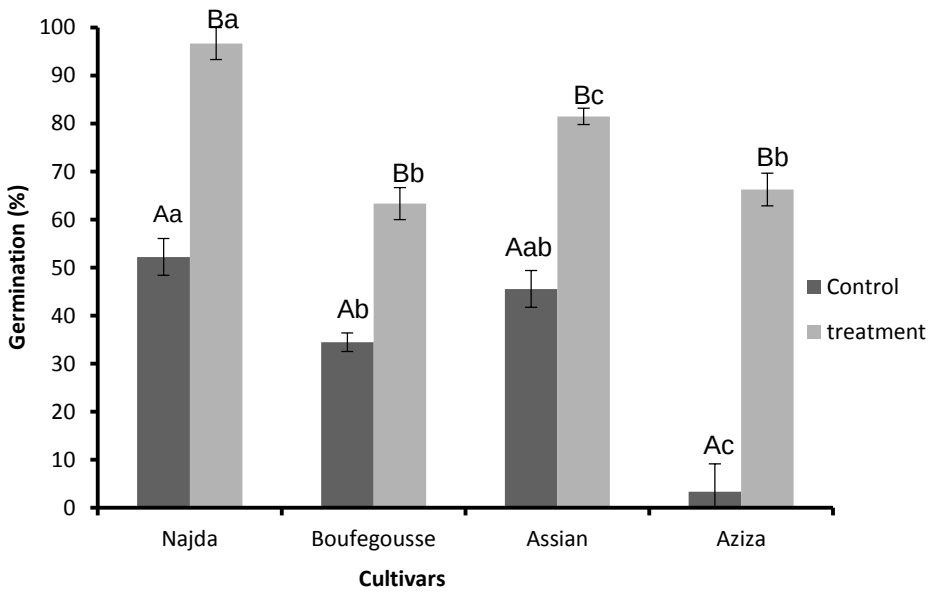

Figure 1: Effect of the treatment on the germination %. Vertical bars represent the standard deviation (n=3). Bars with different capital letters indicate significant differences between treatments in the same cultivar ($p \leq 0.05$). Bars with different small letters indicate significant differences between cultivars in the same treatment ($p \leq 0.05$).

3.3. Effect of the operculum removal on the Germination Speed Index of the tested seeds

The results of the treatment effects on the germination speed index were presented in figure 2. The treatment significantly ($p<0.05$) affected the SIG of the four tested cultivars. SIG of treated seed reached a maximum of 17,944±0,433 for "Najda" seeds compared to 9,295±0,299 for the control seeds, while the minimum value was recorded in "Aziza" seeds 4,869±0,718 compared to 0,351±0,501 for the control. There was a significant difference found between and within groups ($p<0.05$). This treatment improved the speed of the seeds germination; therefore, it helped with the time issue (Figure 2).

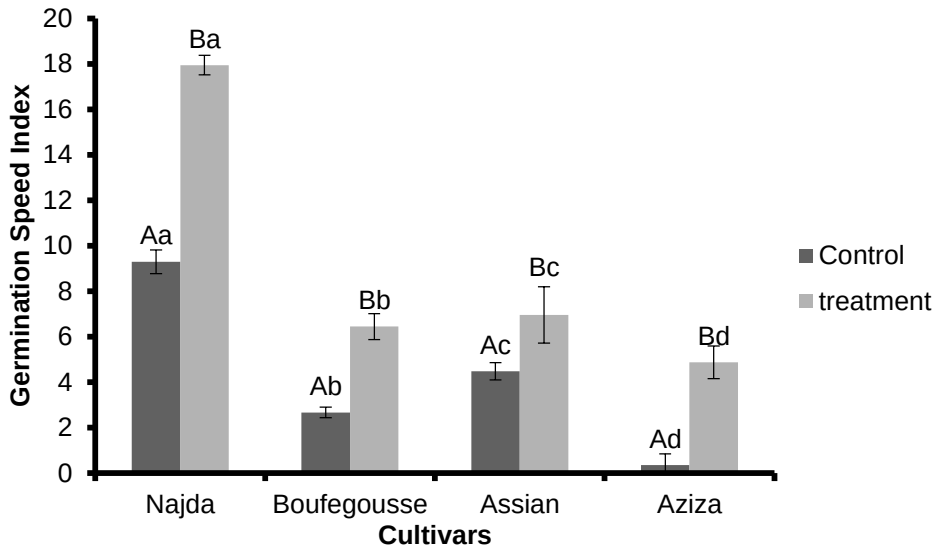

Figure 2: Effect of the treatment on the germination speed index. Vertical bars represent the standard deviation (n=3). Bars with different capital letters indicate significant differences between treatments in the same cultivar ($p \leq 0.05$). Bars with different small letters indicate significant differences between cultivars in the same treatment ($p \leq 0.05$).

3.4. 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.3 represented the

evolution of the germination percentage during 15 days for the treatments as well as the control seeds. The first germinated seed appeared on the fourth day in both control and treatment, for the "Najda" seeds and increased remarkably with time for the treated seeds compared to the control, and this was noticed for the other three cultivars (Boufegousse, Assian, Aziza). "Najda" variety showed the highest germination % reaching 97% in 15 days compared to the control reaching 50% in the same period (Figure 3).

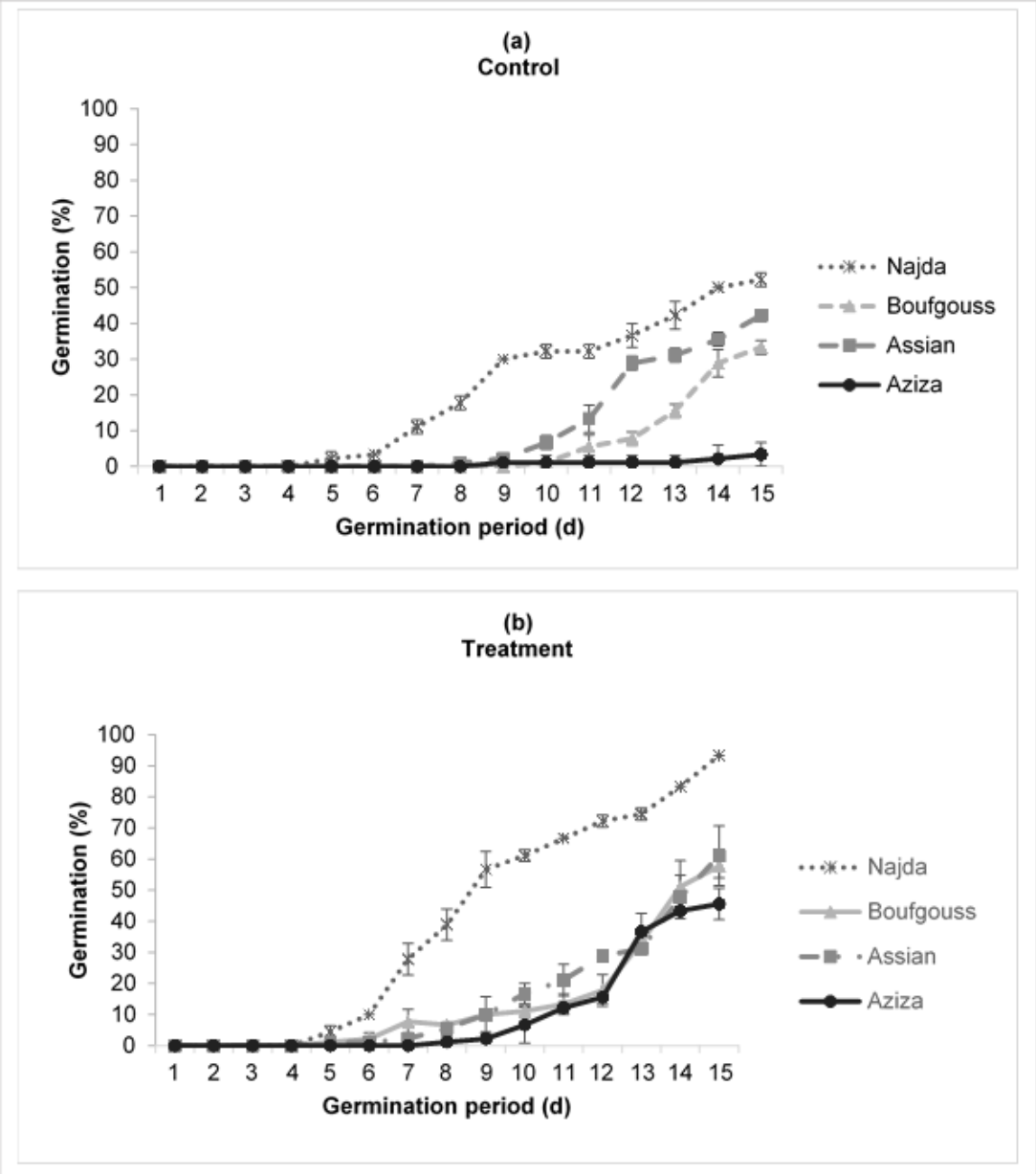

Figure 3: Germination percentage of the control (a) and the treatment (b) seeds at 32 °C for 15 days. Vertical bars represent the standard deviation (n = 30).

4. DISCUSSION

In this study, we reported 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. Seed dormancy is defined as a state in which seeds are prevented from growing even under environmental conditions that are relatively positive for germination [20]. 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 these indices, higher seed germination rates were exhibited by the Najda and Assian seeds (Figure 1) and this can be explained with the formation of certain germination delaying factors during the later stages of date palm seeds development, as reported with some other plant species [17].

The removal of the opercula remarkably enhanced the Najda, Boufegousse, Assian and even Aziza seedlings germination speed index (Figure 2). Abbas et al. (2019) also reported the improvement of the germination rate of the other seedlings by mechanical seed scarification [22]. Thus, it seems that the seed coat of opercula can cause some impermeability to

water and/or gases; therefore, 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 as treated by Mayer and Poljakoff-Mayber (1982) in chapter 4 of the Germination of Seeds [23]. 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 [17].

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 the same aspect treated 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 [24].

Apparently, the removal of the opercula of the "Najda" and "Assian" seeds increased their germination rate to reach a very high level (mean of germination percentage 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.

The hypothesis that the mechanical removal of operculum increases the final percentage and speed of seed germination was fully accepted in "Najda" and "Assian" cultivars, whereas in the case of Boufegousse" and "Aziza" cultivars, it must be questioned. The germination rates exhibited by the similarly treated seeds of these two cultivars were still less than 63.3±1.92 and 61.1±10.18% respectively. This can indicate that certain germination constraints other than those eliminated by the removal of the opercula were still active in the seeds, most likely, these were chemical inhibitors. Working with the seeds of a different cultivar, Warrag and Warrag (2007) reported the presence of such chemicals, which caused the germination process to delay [11]. 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.

However, there was a significant difference in the speed of seed germination between the four cultivars regardless if they were treated or not, based on the Timson's index only (Table 1). Timson (1965) argued that the germination percentage accompanied by information on the time taken for the stated percentage to be reached are unsatisfactory data, and these must be accompanied with the Timson's index for better clearance [21]. Pliszko and Kostrakiewicz-Gierałt (2020) latest article showed similar results in improving the germination speed and percentage of *Solidago* × *niederederi* seeds by mechanical *pappus* removal [25].

5. CONCLUSION

In this study, the pre-germination treatments were most effective on the seed germination. The "Najda" and "Assian", comparing with the "Boufegousse" and the "Aziza" cultivars, exhibited a higher germination rate. The removal of the opercula improved the germination rate of the seeds of all cultivars. The water soaking and the temperature of 32°C brought a further improvement of this parameter. The delay in 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.

Four seedlings compared to the control treatments exhibited a high seed germination rate. 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 germination, due to the damage of the embryos. Therefore, an application of chemical treatment, without damaging the embryos, can be used as another alternative. The processes of improving seed germination can be tiring and sometimes not practical. However, finding new methods that make it less problematic can be a new approach in conserving the biodiversity of these plants and adding new genetic material.

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