Kastamonu Uni., Orman Fakültesi Dergisi, 2022, 22(1): 56-67 Research Article
Kastamonu Univ., Journal of Forestry Faculty
Doi:10.17475/kastorman.1095893

Argan (*Argania spinosa* (L.) Skeels) Seed Germination Under Some Pretreatments of Thermal Shocks

Ouswati SAID ALI [1*], Abdouroihamane HACHEMI[1], Aicha MOUMNI[1], Hamza ZINE[1], Sara ELGADI[1], Tarik BELGHAZI[1], Ahmed OUHAMMOU[1], Abderrahman LAHROUNI[1], Said EL MESSOUSSI[1]

[1] Cadi Ayyad University, Faculty of Sciences Semlalia, Bd Prince Moulay Abdellah, Marrakech, MOROCCO
*Corresponding Author: ouswatisaidali@gmail.com

Received Date: 29.11.2021 Accepted Date: 28.02.2022

Abstract

Aim of study: The objective of this study was to optimize the germination of *A. spinosa* seeds after different physical pretreatments by thermal shock to establish a simple, effective, and less expensive procedure.

Area of study: The study was conducted in the laboratory on seeds from four regions of Morocco (Aoulouz, Essaouira, Sidi Bou Othmane, and Boulaouane)**.**

Material and methods: The seeds were dried and stored. Before the launching of the experimental protocol, they were dehulled and applied each their pre-treatment for the four provenances. The pre-treatment used are the following**.** C: control without thermal shock, HC1: freezing, HC2: hot water, HC3: freezing + hot water.

Main results: The results showed that the onset of germination of argan seeds subjected to pretreatment HC3 was reduced by 2 days on mean compared to seeds that were subjected to pretreatment (C). The application of the cold or hot pretreatment allowed us to reach up to 82% of the final percentage of germination. However, the combination of two pretreatments allowed us to reach up to 100% of the final germination percentage.

Highlights: Freezing combined with hot water significantly improved the germination of *A. spinosa* seeds. This pretreatment could be recommended in the nursery for practitioners.

Keywords: *Argania spinosa,* Seed's Germination, Thermal Shock

Argan (*Argania spinosa* (L.) Skeels) Bazı Termal Şok Ön İşlemleri Altında Tohum Çimlenmesi

Öz

Çalışmanın amacı: Bu çalışmanın amacı, basit, etkili ve daha ucuz bir prosedür oluşturmak için ısı şoku ile farklı fiziksel ön işlemlerden sonra *A. spinosa* tohumlarının çimlenmesini optimize etmektir.

Çalışma alanı: Çalışma Fas'ın dört bölgesinden (Aoulouz, Essaouira, Sidi Bou Othmane ve Boulaouane) tohumlar üzerinde laboratuvarda yürütülmüştür.

Materyal ve yöntem: Tohumlar kurutuldu ve saklandı. Deney protokolünün başlatılmasından önce, kabukları çıkarıldı ve dört orijin için her birine ön muamele uygulandı. Kullanılan ön işlem aşağıdaki gibidir**.** C termal şok olmadan kontrol, HC1: donma, HC2: sıcak su, HC3: donma + sıcak su.

Temel sonuçlar: Sonuçlar, HC3 ön işlemine tabi tutulan argan tohumlarının çimlenme başlangıcının, ön işleme (C) tabi tutulan tohumlara kıyasla ortalama 2 gün azaldığını gösterdi. Soğuk veya sıcak ön işlemin uygulanması, nihai çimlenme yüzdesinin %82'sine kadar ulaşmamızı sağladı. Bununla birlikte, iki ön işlemin kombinasyonu, nihai çimlenme yüzdesinin %100'üne kadar ulaşmamızı sağladı.

Araştırma vurguları: Sıcak su ile birlikte yapılan dondurma işlemi, *A. spinosa* tohumlarının çimlenmesini önemli ölçüde iyileştirmiştir. Bu ön tedavi, fidanlikta uygulayıcılar için önerilebilir.

Anahtar Kelimeler: *Argania spinosa,* Tohum Çimlenmesi, Termal Şok

Introduction

Argan tree (*Argania spinosa* (L.) Skeels) is an endemic species to Morocco (Alouani & Bani-Aameur, 2014) and Algeria (Benaouf et al., 2014) characteristic of arid and semi-arid areas of North Africa (Msanda et al.,

Citation (Atıf): Ali, O. S., Hachemi, A., Moumni, A., Zine, H., Elgadi, S., Belghazi, T., Ouhammou, A., Lahrouni, A., & El Messoussi, S. (2022). Argan (*Argania spinosa* (L.) Skeels) Seed Germination Under Some Pretreatments of Thermal Shocks. *Kastamonu University Journal of Forestry Faculty*, 22 (1), 56-67.

2005). It is characterized by a thermophilic and xerophilic temperament but requires a fairly high atmospheric humidity, hence its presence in areas with a more or less marked oceanic influence (Berka & Harfouche, 2001). Argan forest presents very important socio-economic and ecological interests and source of income for communities, especially rural women (Msanda et al., 2005). The exploitation of argan tree offers also 800 thousand days of jobs and can feed 3 million people (Badaouini, 2015). The argan oil alone (extracted from seeds) is known for its innumerable beneficial effects (Adlouni, 2010). The global argan oil world market has over 100 million USD (Elgadi et al., 2021). The argan tree is also bulwark against desertification phenomena coming from the south (Defaa et al., 2015). The multiple functions of the argan tree have led UNESCO to recognize the Moroccan argan grove as a MAB (Man and the Biosphere) biosphere reserve (Ferradous, 2018). Lastly, the United Nations declared the 10th of May as an international day of the argan tree. However, the overexploitation of the argan tree and the climate change has led to a regression of its surface, supplemented with the rarity of natural regeneration (Defaa et al., 2015; Ferradous, 2018). So, to preserve and promote the sustainability of the current argan ecosystem forest, the only solution is reforestation (Hachemi et al., 2021; Sebbar et al., 2021).

Moreover, argan fruit is a drupe-like fruit in various variable forms: oval, oval apiculate, rounded, globose, drop, fusiform (Bani-Aameur et al., 1999). It is formed of a fleshy pericarp (or pulp) that covers very hard-shelled seeds (or nut) in the center constituting one to three oily, albumenized kernels (Adlouni, 2010). The presence of this very hard tegument, prevents the seed to germinate easily (Berka & Harfouche, 2001; Elmandouri et al., 2020).

Germination stage is the most critical periods of the development of seedlings (Bita et al., 2017). Many tree seeds germinate easily when placed in a favorable temperature and humidity conditions (Dardour et al., 2014). Nevertheless, other species present viable seeds which are unable to germinate, even under the best germination conditions (Dardour et al., 2014). The inability of germination indicates dormancy. To lift tegument inhibitory dormancy and optimize germination, special pretreatments may be necessary (Berka & Harfouche, 2001; Dardour et al., 2014; Elmandouri et al., 2020). According to Guerrouj et al. (2015), often many seeds are wasted for low yields in nurseries due to a lack of knowledge of dormancy by practitioners. It was therefore crucial to simplify the practical methods to dormancy and otherwise optimize germination in the nursery.

Currently, to seed dormancy, studies have used chemical (Bouredja et al., 2011; Dardour et al., 2014) and/or physical pretreatments (Berka & Harfouche, 2001; Dardour et al., 2014; Ferradous, 2018; Gashaw & Michelsen, 2002; McDonnell et al., 2012; Vasques et al., 2014). Studies on the germination of seeds with hard seed coats, such those of *Retama monosperma*, *Stipa tenacissima*, *Grewia coriacea*, *Tamarindus indica*, *Lippia multiflora*, and *Argania spinosa*, favored the use of chemical pretreatments (sulfuric acid, gibberellic acid, hydrogen peroxide, and nitric acid) as the best pretreatment for the removal of integumentary inhibition (Alouani & Bani-Aameur, 2014; Berka & Harfouche, 2001; Bita et al., 2017; Bouredja et al., 2011; Muhammad & Amusa, 2003; Soumahoro et al., 2014). These chemical pretreatments contribute to the softening of the seed coats, thus allowing the germination process (Bita et al., 2017; Bouredja et al., 2011). However, these chemical pretreatments are costly and can be undesirable for the environment and human health (Ghassemi-Golezani et al., 2008).

For physical pretreatments, the use of thermal shock by application of hot (Airi et al., 2009; Azad et al., 2013; Dardour et al., 2014; Guerrouj et al., 2015) or cold water has been initiated (Alouani & Bani-Aameur, 2014; Azad et al., 2013; Gashaw & Michelsen, 2002; McDonnell et al., 2012; Usman et al., 2010; Vasques et al., 2014). Guerrouj et al., (2015) showed that alfalfa (*Medicago arborea*) seeds could lift integumentary inhibition with thermal shocks up to 100°C and 120°C. However, the

pretreatment duration is dependent on the temperature and the seeds characteristics (Bouredja et al., 2011; Mbaye et al., 2002). Many studies have associated cold storage conditions (+4°C) with chemical pretreatments (Alouani & Bani-Aameur, 2014; Elmandouri et al., 2020). These pre-treatments consisted in lifting the integumentary inhibition of argan seeds since the latter does not present a real dormancy problem (Berka & Harfouche, 2001). Nevertheless, information combining two physical pretreatments of different temperatures that could weaken the integument and therefore optimize the germination of argan tree seeds was relatively absent in the literature.

The hypothesis of this study was to demonstrate whether shock temperature (freezing /heat) could optimize the lifting of integumentary inhibition of argan seeds and consequently promote their germination. The objective of this study was to evaluate the germination behavior of argan tree seeds after the application of two pretreatments of thermal shock (freezing at -20°C for 24 hours and hot water (100°C) for 5 minutes) and their combination (freezing at -20°C for 24 hours + hot water (100°C) for 5 minutes), to develop a simple experimental model to optimize the germination of argan tree seeds. The choice of pretreatments was based on high temperatures in order to create a thermal shock capable of lifting the integumentary inhibition imposed by the integuments of the argan seeds without affecting the latter.

Materials and Methods

Plant Material

The seeds used in this study came from ripe argan fruits collected at the end of July 2018 (maturation period) from 4 different regions in Morocco (Aoulouz, Essaouira, Sidi Bou Othmane, and Boulaouane) (Figure 1). The province of Aoulouz (30°37'13.1" N; 8°06'35.2" W) and Essaouira (31°32'07.8" N; 9°28'34.4" W) are in the natural distribution forest area of argan trees. Sidi Bou Othmane (31°96'75.1" N; 7°95'05.9" W) and Boulaouane (32°86'33.6" N; 8°04'23.3" W) are outside of the natural area (Figure 2). The choice was oriented to have climate diversity on the seed's provenances (Figure 2).

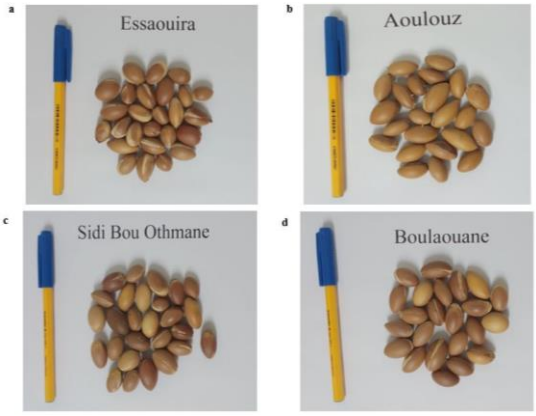

Figure 1. Illustrative image of the four provenances seeds: a. Essaouira, b. Aoulouz, c. Sidi Bou Othmane, and d. Boualouane.

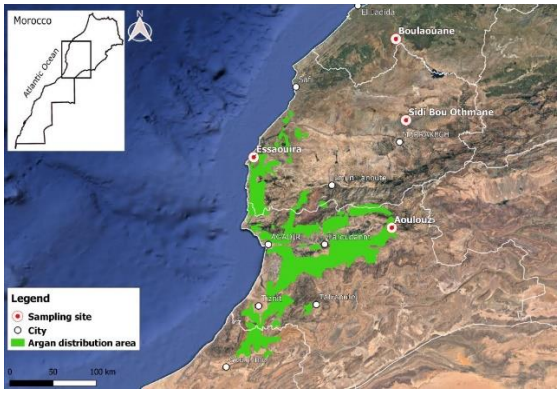

Figure 2. Sampling sites location (Argan forest distribution adopted from (Msanda et al., 2005))

Seed Preparation

After decortication, the argan tree seeds were disinfected. They were soaked in sodium hypochlorite at 16% for 30 seconds and then rinsed thoroughly with top water (Figure 3).

Pretreatment of Seeds and the Experimental Protocol

To remove the integumentary inhibition, three thermal shock pretreatments (HC1, HC2, HC3) and one control without thermal shock (C) were tested on the seeds of each provenance. For each of the pretreatments and the control (Table 1), a batch of 100 seeds divided into 4 was put in Petri dishes containing Whatman paper hydrated daily for maintaining humidity (25 seeds per Petri box).

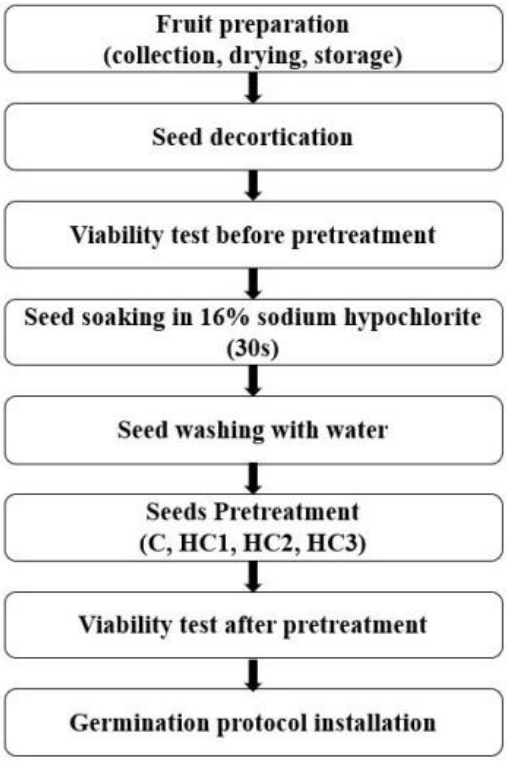

Figure 3. Diagram of the experimental and methodological approach adopted in work.

Table 1. Pretreatments applied to argan seeds (C: control without thermal shock, HC1: freezing, HC2: hot water, HC3: freezing + hot water)

	Pretreatment codes			
	C	HC1	HC2	HC3
Freezing (-20°C) for 24h	-	✓	-	✓
Hot water (100°C) for 5 min	-	-	✓	✓
Water at room temperature (24h)	✓	✓	✓	✓

Seed Characteristics

Seed's characterization was carried out to be able to inquire about the morphological quality of the seeds. It concerns the weight of the seeds, the kernels, and the moisture content. The weight was measured (100 seeds per repetition and per province) with an analytical balance (KERN) and the seed moisture content (%) was calculated using the following formula (Montaño-Arias et al., 2021):

$$MC\ (\%) = \left(\frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{FW}}\right) \times 100 \qquad (1)$$

The fresh weight "FW" was obtained with an analytical balance (KERN), from three replicates of 10 seeds per provenance. Then, seeds were placed in an incubator (BINDER Model ED 56) at 105 °C until a constant weight was achieved, to obtain the dry weight "DW".

Tetrazolium Viability Test

The tetrazolium viability test was applied to argan seeds before and after pretreatment according to the method described by Ferradous (2018). The purpose of this test was to confirm the viability of the seeds even after having undergone the thermal shock. The kernels were separated longitudinally with a sharp blade and then dipped in the tetrazolium solution (0.2%) in Petri dishes and incubated for 3 hours at 40°C with 25 kernels per Petri dish. After staining, the kernels were washed under tap water to remove excess dye.

Germination Characteristics

The seed is considered as germinated once the radicle pierces the integument. The germinated seeds were counted from the day the experimentation began. For the germination, different variables were monitored namely: latent period (LP), final percentage germination (FPG), mean germination time (MGT), and germination rate (GR) (Ferradous, 2018; Montaño-Arias et al., 2021; Mwendwa et al., 2020).

-Latent period (LP): is the time that elapses from the start of the test to the first germination;

-Final germination percentage (FGP): represents the number of seeds germinated (N_g) over the total number of seeds started to germinate (N_t):

$$\mathrm{FGP}\ (\%) = \frac{N_g}{N_t} \times 100 \qquad (2)$$

-Mean germination time (MGT): corresponds to the elapsed between initial germination set up and the last germination:

$$MGT = (\sum_{k=0}^{i} n_i * t_i)/(\sum_{k=0}^{i} n_i) \quad (3)$$

where n_i is the number of germinated seeds and t_i is the germination time.

-Germination rate (GR) was calculated as follows:

$$GR = \sum_{k=0}^{i}(N_i)/\mathrm{t} \quad (4)$$

where N_i is the number of germinated seeds in 1 day (i); t is the time between the sowing and the germination of the last seed.

Statistical Analysis

Data were subjected by a two-way ANOVA test ($p<0.05$), followed by a comparison of means test (Tukey's HSD, $p<0.05$) to evaluate the effects of provenances and pretreatments. One-way analysis of variance (ANOVA) was performed to analyze seed germination variables data over the different pretreatments. Multiple pairwise means comparisons were then conducted using Tukey's range test to separate means. A Principal Component Analysis (PCA) was performed to determine the thermal shock that will give the best response for seed germination. All analyses were performed using R Studio software.

Results

Seed Characteristics and Tetrazolium Viability Test

The weight of seed and kernel varied significantly ($p<0.001$) with the different provenances (Table 2). However, no significant difference was observed in moisture content. The seed viability test with tetrazolium (0.2%) showed that the applied thermal shocks did not affect them (Figure 4).

Table 2. Seed's characterization of argan tree

Provenances	Weight of seeds (g)	Weight of kernels (g)	Moisture content (%)
Aoulouz	279.12 ± 0.14d	27.16 ± 0.51a	4.48 ± 0.33a
Essaouira	230.13 ± 0.11b	31.24 ± 0.86b	6.34 ± 2.65a
Sidi Bou Othmane	258.98 ± 0.54c	27.40 ± 0.25a	4.31 ± 0.07a
Boulaouane	219.92 ± 0.22a	30.34 ± 0.19b	6.38 ± 0.37a
p-value	<0.001	<0.001	0.175
F-value	25003	45.48	2.13

Vertically, the means followed by the different letters are significantly different at a threshold of 5%.

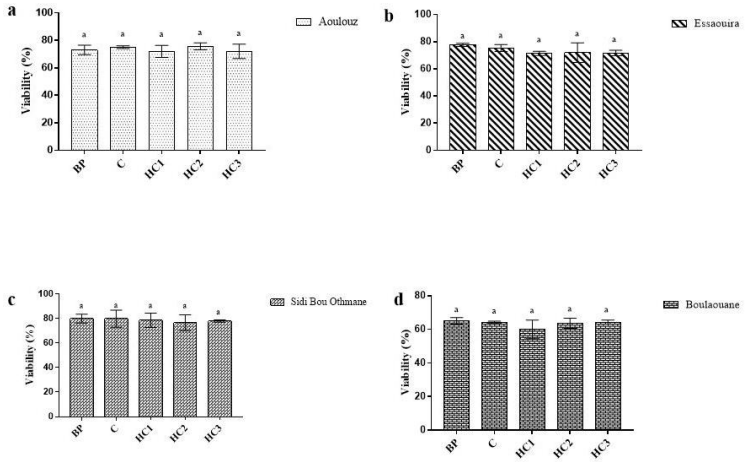

Figure 4. Viability of argan seeds assessed via tetrazolium test (soaking in a 0.2% Tetrazolium solution and incubating for 3 hours at 40°C) before and after application of pretreatments. Means followed by the same letter do not differ, according to the Tukey's Post Hoc at a 5% probability. BP: before pretreatment, C: control (no thermal shock), HC1: freezing, HC2: hot water, HC3: freezing + hot water. a. Aoulouz provenance, b. Essaouira provenance, c. Sidi Bou Othmane provenance, and d. Boualouane provenance.

Effects of Provenance and Pretreatment of Seed Germination

Provenances, pretreatments, and their interaction are affected significantly by all the germination variables (FGP, GR, and MGT) except for LP. However, a significant effect was observed after the use of pretreatments (Table 3).

Table 3. Result of the two-way ANOVA for analysis of the effect of argan seed provenance on final percentage germination (FPG), latent period (LP), mean germination time (MGT) saturation rate (SR) and germination rate (GR)

		FPG (%)	LP (days)	MGT (days)	GR (seed/days)
	DF	F	F	F	F
Provenance	3	153.58***	2.22 ns	261.13***	49.64***
Pretreatment	3	58.34***	8.44***	24.22***	19.59***
Provenance and pretreatment	9	10.61***	1.03 ns	14.12***	3.30**

ns=not significant; *p <0.05; **p<0.01; ***p<0.001; DF=degrees of freedom; F=factor; all treatments were made in quadruplicate

Freezing and Hot Water Pretreatment Effects

The effect of used pretreatments for germination of argan seeds had a significant effect on most variables monitored, as shown in Tab. 4. FGP and GR showed significant differences across all provenances except for Boulaouane (Table 4). Nevertheless, the use of the combination of freezing and hot water (HC3) gave higher germination percentages and germination rates for all provenances in comparison to the control as well as freezing (-20°C) (HC1) and the application of hot water (100°C) (HC2). The final percentages of germination are 75%, 81%, 96% and 100% respectively for the provenances of Boulaouane, Essaouira, Aoulouz and Sidi Bou Othmane. In addition to the FGP, the combination of freezing (-20°C) and hot water (100°C) also reduced the mean germination time to 12.94, 13.57 and 14.21 days respectively for the Sidi Bou Othmane, Boulaouane and Essaouira provenances, except for of Aoulouz provenance where the differences were not significant within the pretreatments. The evaluation of LP gave insignificant differences for all of used provenances except for the provenance of Aoulouz. However, the results of the combination of freezing (-20°C) and hot water (100°C) gave very short latent periods (3 days for Boulaouane and 2 days for Sidi Bou Othmane, Aoulouz, and Essaouira).

Principal Component Analysis (PCA)

The application of PCA is essentially based on the use of the mean values of data to establish similarities between different pretreatments and different variables for all provenances (Figure 5). PCA revealed that the two first components explain 79.10% of the variability in data, being 54.10% for dimension 1 (Dim1) and 25.00% for dimension 2 (Dim2). The variables FGP and GR were positively correlated to the first dimension (Dim1). The LP variable is negatively correlated to the first dimension (Dim1). The MGT is positively correlated to the second dimension (Dim2). The FGP and GR variables are strongly positively correlated to each other (Figure 5a). PCA showed that across all provenances, the HC3 pretreatment shows a stronger FGP and GR than the others (Figure 5b).

Table 4. Mean values of final germination percentage (FGP), mean germination time (MGT), germination rate (GR), and latent period (LP) of argan seeds under different germination pretreatments for the provenances of Sidi Bou Othmane, Boulaouane, Aoulouz, and Essaouira.

Provenance	Pretreatment	FGP (%)	MGT (days)	GR (seeds/days)	LP (days)
Aoulouz	C	87 ± 1.89a	16.54 ± 0.42a	4.14 ± 0.17a	4 ± 0.81b
	HC1	92 ± 3.54ab	16.87 ± 0.42a	4.38 ± 0.11ab	4 ± 0.81b
	HC2	88 ± 7.36ab	16.26 ± 0.15a	4.19 ± 0.14a	3 ± 0.81ab
	HC3	96 ± 0.81b	16.67 ± 0.23a	4.57 ± 0.14b	2 ± 0.81a
	p-value	*	ns	**	*
	F-value	3.81	2.38	7.13	5.5
Essaouira	C	57 ± 1.77a	16.70 ± 0.25d	2.71 ± 0.47a	3 ± 0.00a
	HC1	60 ± 4.99a	16.18 ± 0.31c	2.86 ± 0.20a	3 ± 0.81a
	HC2	79 ± 0.81b	15.69 ± 0.16b	3.71 ± 0.08b	3 ± 0.85a
	HC3	81 ± 1.41b	14.21 ± 0.09c	3.86 ± 0.13b	2 ± 0.81a
	p-value	***	***	***	ns
	F-value	81.30	92.41	18.83	2.00
Sidi Bou Othmane	C	79 ± 0.81a	13.39 ± 0.08ab	3.76 ± 0.66a	4 ± 0.81b
	HC1	82 ± 1.00a	12.98 ± 0.49ab	3.90 ± 0.08ab	3 ± 1.41ab
	HC2	81 ± 3.36a	13.85 ± 0.44b	3.86 ± 0.56ab	3 ± 0.00ab
	HC3	100 ± 0.00b	12.94 ± 0.53a	4.76 ± 0.06b	2 ± 0.81a
	p-value	***	*	*	ns
	F-value	53.02	3.91	4.40	3.20
Boulaouane	C	66 ± 3.26a	13.86 ± 0.08a	3.14 ± 0.25a	4 ± 0.81a
	HC1	69 ± 3.36ab	15.57 ± 0.31b	3.29 ± 0.16a	3 ± 1.41a
	HC2	69 ± 5.47ab	14.13 ± 0.27a	3.45 ± 0.22a	4 ± 0.81a
	HC3	75 ± 4.24b	13.57 ± 0.73a	3.57 ± 0.25a	3 ± 0.81a
	p-value	ns	***	ns	ns
	F-value	3.26	17.41	2.66	1.33

C: control (no thermal shock), HC1: freezing, HC2: hot water, HC3: freezing + hot water, ns: no significant, **and ***: significant at p≤0.01 and p≤0.001. Values with the same letter are not significantly different at p<0.05. Data represent means ± SE (n = 4).

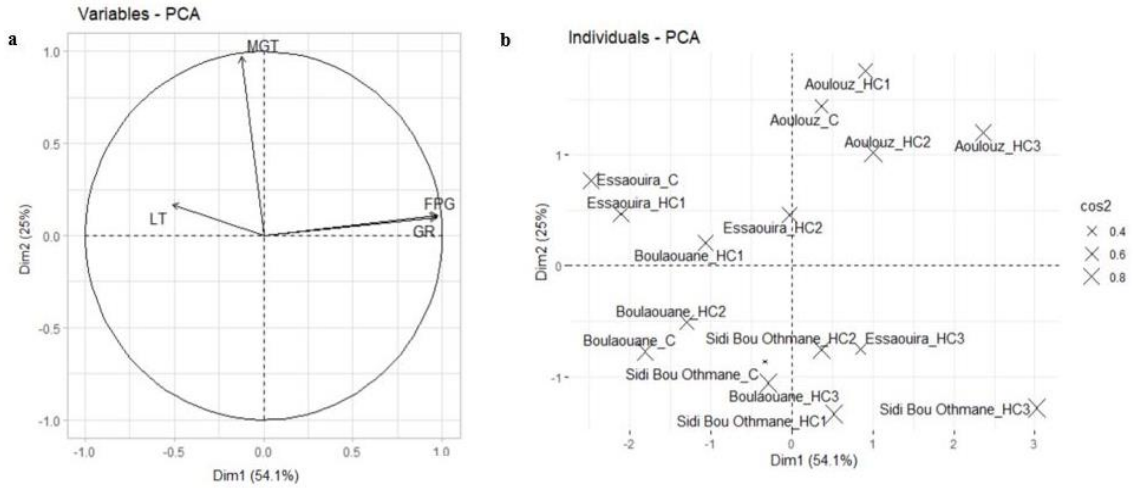

Figure 5. Principal Component Analysis (PCA): **a.** Representation of germination variables (final germination percentage (FGP), mean germination time (MGT), germination rate (GR), and latent period (LP)) according to principal component analysis. **b.** Representation of pretreatments and provenances according to the principal component analysis. Dimensions 1 and 2 represent the principal components of the PCA. The latter is responsible for determining two axes that best explain the dispersion of the object, interpreted as a point cloud. It is also responsible for the explained inertial scheduling, the second axis being perpendicular to the first one. C: control (no thermal shock), HC1: freezing, HC2: hot water, HC3: freezing + hot water.

Discussion

Many researchers, have discussed germination techniques for different species to seed dormancy by applying different pretreatments, which could improve the germination rate and accelerate the germination process (Airi et al., 2009; Alouani & Bani-Aameur, 2014; Azad et al., 2013; Dardour et al., 2014; Üçler et al., 2017). However, physical seed dormancy is a serious problem in seed germination (Schmidt, 2000). Pretreatments before sowing have been shown to improve the germination of seeds with a hard and solid seed coat (Hossain et al., 2005). Indeed, the tegument once cracked facilitates the humidification of the almond and starts the germination.

In the present study, the application of pretreatment HC1 (freezing) or HC2 (hot water) gave satisfactory results on the germination of argan tree seeds. However, although these pretreatments have better final germination the percentages and mean germination times compared to the control, showed no significant difference was reported. This lack of difference can be explained by the influence of germination temperature (Berka & Harfouche, 2001). A temperature of +28°C improves the germination of argan tree seeds. The ambient temperature seems to be an important factor in the germination of Argan seeds (Berka & Harfouche, 2001). The HC2 pretreatment (hot water) improved germination with a minimum germination percentage of 69% and a maximum of 88%. Some studies have shown that the use of hot water (100°C) as a pretreatment at a duration not exceeding 6 minutes allowed to obtain significant germination (Dardour et al., 2014; Guerrouj et al., 2015). Guerrouj et al. (2015) also revealed that hot water pretreatment (100°C for 4 and 6 min) of *Medicago arborea* gave better germination success (70%). Similar to HC2 pretreatment, HC1 pretreatment (freezing) improved seed germination. Nevertheless, compared to the control and HC2 pretreatment, no significant difference ($p>0.05$) was reported on all the evaluated variables. Similar results were reported by Martinková and Honěk (2007). Indeed, freezing (-20°C) seeds for 5 days did not affect the final germination percentage (Martinková & Honěk, 2007). These results prove that freezing (-20°C) preserves seed quality. The tetrazolium test used in this study showed no difference in viability for all tested pretreatments.

Although the HC1 and HC2 pretreatment performed well on germination, the combination of these two pretreatments (HC3) showed a significant difference compared to the other pretreatments. The combination of freezing and hot water (HC3) increased the final germination percentage and reduced the mean germination time compared to the other pretreatments. This, might be due to the effect of HC3 pretreatment that cause the rapid seed coat cracking, allowing it to soften and sufficient water and oxygen to pass into the seed (Dardour et al., 2014). This pretreatment was also able to avoid long exposure of the seeds to contamination. Alouani & Bani-Aameur (2014) have shown that rapid germination of seeds avoids contamination.

The principal component analysis applied on the pretreatments and provenances to the studied variabilities corroborates these results. It has been reported that storing *Lupinus albus* L. and *Trifolium pratense* L. seeds in a low temperature of -80°C and then thawing them immediately in hot water (90°C) improves their germination (Tiryaki & Topu, 2014). In fact, according to the same authors, the cracks on the coat are the result of the thermal shock expansion or contraction of the coat on the freezing seed. Depending on the species, seed coat thickness, and seed size used, it is possible to hear a hatching sound when the seeds are immersed in hot water after storage in a deep freezer (Tiryaki & Topu, 2014). However, a previous study reported that alfalfa seeds, subjected to freeze-thaw pretreatment (cooling temperatures to -5°C or -15°C for 36 h and warming to room temperature for six days), did not improve germination (Midgley, 1926). Similarly, freeze-thaw pretreatment of dairy vetch seeds at -22°C for 2, 4, 7, 30, 60, 90, or 180 days did not improve seed germination by more than 78% (Shibata & Hatakeyama, 1995).

On the other hand, the present study showed a significant effect of the studied

variables on seed provenance. Indeed, the percentage of final germination, mean germination time, and germination rate of the variable were significantly differentiated according to the provenance of the seeds except for the latency time. Similar results of the influence of the provenance on the germination power of argan seeds were shown by Ferradous (2018). The study also revealed that the interaction between provenances and treatments had a significant effect ($p<0.01$) on final germination percentage, mean germination time, and germination rate. In addition, the argan seeds and kernels weights evaluated on the characterization of seeds showed significant differences in relation to the provenance. Similar significant differences to those observed by Ferradous (2018) on the provenance are recorded on the argan seeds and kernels weights. These observations on the weight could be related to climatic conditions (Ferradous, 2018; Medrano et al., 2003). However, the changes that affected the weights of argan seeds and kernels did not affect the percentage of moisture content. These results corroborate those of the study of Ferradous (2018), which also showed that the argan seed weight is not related to moisture content. Delgado et al. (2001) also demonstrated that seed weight is not related to seed quality. This lack of correlation between seed weight and seed quality (measured as germination probability) may be a consequence of the fact that seed weight is not related to embryonic vitality or reserve quantity (Delgado et al., 2001; Ferradous, 2018).

Conclusion

The present study has proposed to evaluate the germination behavior of argan seeds after the application of two pretreatments of thermal shock (freezing at -20°C for 24 hours and hot water (100°C) for 5 minutes) and their combination (freezing at -20°C for 24 hours + hot water (100°C) for 5 minutes). The results showed that the combination of freezing (-20°C) and hot water (100°C) of the argan seeds have an optimal, uniform, and fast germination of argan seeds. The pretreatment by the combination of cold (-20°C) and hot water (100°C) is the fastest and least efficient method to release the dormancy of the argan seeds imposed by the hardness of the tegument. This pretreatment is an asset for other plant species that represent an integumentary dormancy.

Acknowledgments

This work was supported by funding from the Regional Center for Forestry Research of Marrakesh, Morocco. The authors would like to acknowledge the Regional Center for Forestry Research of Marrakesh, others persons (colleagues, Ph.D students, professor Kenza Loutfi), and the anonymous reviewers for their suggestions and comments that improved the content of this article.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: O. S. A., H. A., S. E. M.; Investigation: O. S. A., H. A., T. B.; Material and Methodology: O. S. A., H. A.; Supervision: T. B., S. E. M.; Visualization: A. O., A. L., ; Writing-Original Draft: O. S. A.; Writing-review & Editing: O. S. A., H. A., T. B., A. O., A. L., S. B., H. Z. A. M.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

Funding

The authors declared that this study has received no financial support.

References

Adlouni, A. (2010). Argan oil: From nutrition to health. *Phytotherapie*, 8(2), 89–97. https://doi.org/10.1007/s10298-010-0538-9.

Airi, S., Bhatt, I. D., Bhatt, A., Rawal, R. S. & Dhar, U. (2009). Variations in seed germination of *Hippophae salicifolia* with different presoaking treatments. *Journal of Forestry Research*, 20(1), 27–30. https://doi.org/10.1007/s11676-009-0005-3.

Alouani, M. & Bani-Aameur, F. (2014). Viabilité

et vieillissement des semences d'arganier (*Argania spinosa* (L.) Skeels. *Afrique Science: Revue Internationale Des Sciences et Technologie*, 10(1), 245–255.

Aravind, J., Vimala Devi, S., Radhamani, J., Jacob, S. R. & Srinivasan, K. (2019). The germinationmetrics Package: A Brief Introduction. In *ICAR-National Bureau of Plant Genetic Resources, New Delhi.* (p. 46). https://doi.org/10.5281/zenodo.1219630.

Azad, M. S., Nahar, N. & Matin, M. A. (2013). Effects of variation in seed sources and pre-sowing treatments on seed germination of *Tamarindus indica*: A multi-purpose tree species in Bangladesh. *Forest Science and Practice*, 15(2), 121–129. https://doi.org/10.1007/s11632-013-0211-0.

Badaouini, M. (2015). *Contribution to the selection and vegetative reproduction by grafting of performing argan* (*Argania spinosa* (L) Skeels.) Specimen. In PhD thesis, IAV Hassan II, Agadir, Morocco.

Bani-Aameur, F., Ferradous, A. & Dupuis, P. (1999). Typology of fruit and stones of *Argania spinosa* (Sapotaceae). *Forest Genetics*, 6(4), 213–219.

Benaouf, Z., Miloudi, A. & Belkhodja, M. (2014). Germination tests of seeds of argan tree (*Argania spinosa* (l.) skeels) of two sources (Tindouf and Mostaganem) in the semi-arid western Algerian. *African Journal of Plant Science*, 8(6), 260–270. https://doi.org/10.5897/ajps2014.1171.

Berka, S. & Harfouche, A. (2001). Effets de quelques traitements physico-chimiques et de la température sur la faculté germinative de la graine d'arganier. *Biologie et Ecologie*, 5–10.

Bita, A. M., Mpika, J., Saya, R. A., Ngantsoue, L. & Attibayeba, P. (2017). Evaluation des conditions de germination des noyaux de *Grewia coriacea* Mast. (Malvaceae). *International Journal of Biological and Chemical Sciences*, 11, 2809–2825.

Bouredja, N., Mehdadi, Z., Bendimered, F. Z. & Chérifi, K. (2011). Effets de quelques prétraitements physico—chimiques sur la levée de l'inhibition tégumentaire des graines de *Retama monosperma* Boiss. et recherches des conditions thermiques optimales de germination. *Acta Botanica Gallica*, 158(4), 633–643. https://doi.org/10.1080/12538078.2011.10516300.

Dardour, M., Daroui, E. A., Boukroute, A., Kouddane, N. & Berrichi, A. (2014). Etude de prétraitements des graines de *Brachychiton populneus* (Schott & Endl.) R.Br. et *B. acerifolius* F.Muell. en faveur de leur germination. *Journal of Materials and Environmental Science*, 5(6), 1877–1884.

Defaa, C., Elantry, S., El Alami, S. L., Achour, A., El Mousadik, A. & Msanda, F. (2015). Effects of Tree Shelters on the Survival and Growth of *Argania spinosa* Seedlings in Mediterranean Arid Environment. *International Journal of Ecology*, https://doi.org/10.1155/2015/124075.

Delgado, J. A., Serrano, J. M., López, F. & Acosta, F. J. (2001). Heat shock, mass-dependent germination, and seed yield as related components of fitness in *Cistus ladanifer*. *Environmental and Experimental Botany*, 46(1), 11–20. https://doi.org/10.1016/S0098-8472(01)00076-4.

Elgadi, S., Ouhammou, A., Taous, F., Zine, H., Papazoglou, E. G., Elghali, T., Amenzou, N., El Allali, H., Aitlhaj, A. & El Antari, A. (2021). Combination of stable isotopes and fatty acid composition for geographical origin discrimination of one argan oil vintage. *Foods*, 10(6), 1–13. https://doi.org/10.3390/foods10061274.

Elmandouri, F. Z., Fadli, A., Talha, A., Chetto, O., Omar, A., El Bahloul, Y., Benkirane, R. & Benyahia, H. (2020). Development of optimal conditions for the germination of argan seeds (*Argania spinosa* (L.) Skeels). *Plant Cell Biotechnology and Molecular Biology*, 21(37–38), 57–66.

Ferradous, A. (2018). *Optimisation de la production de plants d ' arganier* (*Argania spinosa* (L .) Skeels) *en pépinière*, (PhD thesis, Faculty of Science Semlalia Marrakesh, Cadi Ayyad University).

Gashaw, M. & Michelsen, A. (2002). Influence of heat shock on seed germination of plants from regularly burnt savanna woodlands and grasslands in Ethiopia. *Plant Ecology*, 159(1), 83–93. https://doi.org/10.1023/A:1015536900330.

Ghassemi-Golezani, K., Sheikhzadeh-Mosaddegh, P. & Valizadeh, M. (2008). Effects of hydro-priming duration and limited irrigation on field performance of chickpea. *Research Journal of Seed Science*, 1(1), 34–40.

Guerrouj, K., Bouterfas, M., Abdelmoumen, H., Boukroute, A. & Missbah El Idrissi, M. (2015). Prétraitement des graines de la luzerne arborescente (*Medicago arborea* L .) et influence de la salinité et de la température sur leurs germinations. *Nature & Technology*, *Juin*, 41–46.

Hachemi, A., Said Ali, O., Belghazi, T., Lahrouni, A., El Mercht, S., El Hassan, C. &

El Messoussi, S. (2021). Effect of hydric and light stress on biomass, nutrient uptake and enzymatic antioxidants of *Argania spinosa* seedlings. *Archives of Biological Sciences*, 73(1), 145–153. https://doi.org/10.2298/ABS201220010H.

Hossain, M. A., Arefin, M. K., Khan, B. M. & Rahman, M. A. (2005). Effects of Seed Treatments on Germination and Seedling Growth Attributes of Horitaki (*Terminalia chebula* Retz.) in the nursery. *Research Journal of Agriculture and Biological Sciences*, 1(2), 135–141. http://www.resecol.wur.nl/gest/privateGEST/Asia/Papers/Hossain 2005 seed germination.pdf.

Martinková, Z. & Honěk, A. (2007). The effect of cryopreservation on germination of dandelion seeds. *Plant Protection Science*, 43(2), 63–67. https://doi.org/10.17221/2257-pps.

Mbaye, N., Diop, A. T., Gueye, M., Diallo, A. T., Sall, C. E. & Samb, P. I. (2002). Etude du comportement germinatif et essais de levée de l'inhibition tégumentaire des graines de *Zornia glochidiata* Reichb. ex DC., légumineuse fourragère. *Revue d'élevage et de Médecine Vétérinaire Des Pays Tropicaux*, 55(1), 47–52. https://doi.org/10.19182/remvt.9845.

McDonnell, A., Grant, M. & Coons, J. (2012). Effects of Hot Water on Breaking Seed Dormancy of the Endangered Kankakee Mallow (*Iliamna remota* Greene (Malvaceae). *Erigenia*, 25, 8–13. http://thekeep.eiu.edu/bio_fac/192

Medrano, H., Escalona, J. M., Cifre, J., Bota, J. & Flexas, J. (2003). A ten-year study on the physiology of two Spanish grapevine cultivars under field conditions: Effects of water availability from leaf photosynthesis to grape yield and quality. *Functional Plant Biology*, 30(6), 607–619. https://doi.org/10.1071/FP02110.

Midgley, A. R. (1926). Effect of alternate freezing and thawing on the impermeability of alfalfa and dodder seeds. *Journal of the American Society of Agronomy*, 18, 1087–1098.

Montaño-Arias, S. A., Zavaleta-Mancera, H. A., Camargo-Ricalde, S. L. & Grether, R. (2021). Effect of seed age on germination, seedling survival and growth of *Mimosa luisana* (Leguminosae). *Trees - Structure and Function*, *35*(1), 231–239. https://doi.org/10.1007/s00468-020-02031-5.

Msanda, F., El Aboudi, A. & Peltier, J.-P. (2005). Biodiversité et biogéographie de l'arganeraie marocaine. *Agricultures (Montrouge)*, 14(4), 357–364.

Muhammad, S. & Amusa, N. (2003). Effects of sulphuric acid and hot water treatments on seed germination of tamarind (*Tamarindus indica* L). *African Journal of Biotechnology*, 2(9), 276–279. https://doi.org/10.5897/ajb2003.000-1056.

Mwendwa, B. A., Kilawe, C. J. & Treydte, A. C. (2020). Effect of seasonality and light levels on seed germination of the invasive tree Maesopsis eminii in Amani Nature Forest Reserve, Tanzania. *Global Ecology and Conservation*, 21, 1–11. https://doi.org/10.1016/j.gecco.2019.e00807

Schmidt, L. H. (2000). Guide to handling of tropical and subtropical forest seed. In *Danida Forest Seed Centre* (Issue 2000). http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.706.5441&rep=rep1&type=pdf.

Sebbar, B., Moumni, A., Lahrouni, A., Chehbouni, A., Belghazi, T. & Maksoudi, B. (2021). Remotely sensed phenology monitoring and Land-cover classification for the localization of the endemic argan tree in the southern-west of Morocco. *Journal of Sustainable Forestry*, 1–15.

Shibata, T. & Hatakeyama, Y. (1995). Breaking of Dormancy in the Seeds of *Astragalus mongholicus* Bunge (Leguminosae). *Journal of Plant Physiology*, 146(3), 366–368. https://doi.org/10.1016/S0176-1617(11)82070-5.

Soumahoro, A., Kone, T., Kone, M., Konate, S., Kouadio, J. & Zouzou, M. (2014). Etablissement d'un protocole efficace de germination des graines du thé de savane (*Lippia multiflora* Mold., Verbenaceae). *Agronomie Africaine*, 26(2), 137–146.

Tiryaki, I. & Topu, M. (2014). A novel method to overcome coat-imposed seed dormancy in *Lupinus albus* L. and *Trifolium pratense* L. *Journal of Botany*, 2014, 1–6. https://doi.org/10.1155/2014/647469.

Üçler, A., Acar, C., Yücesan, Z. & Oktan, E. (2017). Effect of thermal pretreatment on germination of seeds from different provenances of subalpine oriental spruce (*Picea orientalis* (L.) Link.) forest in Turkey. *Journal of Sustainable Forestry*, 37(3), 302–315. https://doi.org/10.1080/10549811.2017.1406806.

Usman, A., Sotannde, O. A., Mbaya, Y. P. & Musa, Y. (2010). Effects of Hot and Cold Water Pre-Treatments on Emergence of Acacia Senegal Seeds in the Nursery. *Journal of Research in Forestry, Wildlife and Environment*, 2(2), 207-213–213.

Vasques, A., Vallejo, V. R., Santos, M. C. & Keizer, J. J. (2014). The role of cold storage and seed source in the germination of three Mediterranean shrub species with contrasting dormancy types. *Annals of Forest Science*, *71*(8), 863–872. https://doi.org/10.1007/s13595-014-0395-z.