Effects of the water presoaking duration and gibberellic acid treatments on seed germination of Argania spinosa L. under nursery conditions

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EFFECTS OF THE WATER PRESOAKING DURATION AND GIBBERELLIC ACID TREATMENTS ON SEED GERMINATION OF *Argania spinosa* L. UNDER NURSERY CONDITIONS

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ABSTRACT

In this study, the effects of pre-sowing soaking seed treatments and application of different gibberellic acid (GA_3) solutions on the germination of argan (*Argania spinosa* L.) were studied. Argan seeds were exposed to 24, 48, 72 and 96 h pre-sowing soaking and 0, 250, 500, 1000 and 2000 mg L^{-1} GA_3 treatments. The highest germination rate (56.67%) in argan seeds soaked prior to sowing was obtained with soaking in water for 96 h. The germination rate increased by 100% in argan seeds soaked for 96 h than seeds soaked for 24 h. In parallel with the increase in GA_3 dose, germination rate of argan seeds increased, as well. The 2000 ppm GA_3 was the best treatment (58.34%). Soaking seeds in water for 96 h or 2000 ppm GA_3 practices reduced the germination time of argan seeds to reach 50% of germination. The highest germination rate occurred on the 22^{nd}-24^{th} days with the soaking practices of seeds prior to sowing, while it took place on the 20^{th}-22^{th} days with GA_3 practices.

KEYWORDS: *Argania spinosa*, germination, gibberellic acid, dormancy, seed soaking

1. INTRODUCTION

Argan is a species indigenous to semi-arid regions comprising a great area between Essaouria and Agadir in southwest Morocco [1]. Its tree is 8-10 m high, with small leaves and thorns, and can live for 150-200 years. Berbers, the indigenous people of Morocco, meet many of their needs from the argan plant: quite valuable oil extracted from its solid fruit seeds is used in cosmetic and medical fields, and its wood is used as firewood or in furniture business. Its leaves and fruits are used for feeding goats and camels [2]. Argan tree has a deep root and, thanks to this feature, they are used for preventing erosion and controlling desertification. Argan tree can be easily grown in arid and semi-arid climate areas receiving less than 200 mm of annual precipitation [1]. As it does not require much irrigation and can well adapt to brackish irrigation, in recent years attempts have been intensified to grow this plant in desert areas with lack of irrigation possibility, in the neighboring countries of Morocco (Algeria, Tunisia) as well as some other noncontiguous countries (Kuwait, Israel, Australia, Mexico).

The southeast parts of Turkey have similar climate conditions to southwest Morocco, where argan trees grow naturally. Şanlıurfa is a province located in southeast Turkey with 300-400 mm of annual rainfall and 18.584 km^2 of surface area. Şanlıurfa is the warmest and most arid province with lowest rainfall rate in Turkey during summer. Argan can be successfully grown in southwest Morocco, where there is no possibility for irrigation and only a small rainfall amount, and this feature has attracted the attention of Turkish entrepreneurs visiting Morocco because of touristic purposes. They wonder if argan tree can be grown in Şanlıurfa Province in southeast Turkey, which has similar climate conditions to Morocco. This study is carried out to investigate whether argan plantations can be established in Şanlıurfa and neighboring provinces using the seedlings obtained from the germination of argan seeds brought by Turkish businessmen.

Propagation of argan plants with seeds and cuttings is quite difficult. Scientific research on argan seed germination and seedling establishment is new and limited [3]. Conventional propagation of *Argania spinosa* is most commonly from seed. However, the seeds of *Argania spinosa* exhibit dormancy [4], and their germination is not always ensured. Argan seeds shell is quite thick and hard. Four treatments have proven to be satisfactory for overcoming argan seed coat impermeability: mechanical scarification; cracking of hard seed coat, immersion in sulphuric acid, or soaking in hot water [4, 5].

Al-Manaie *et al.* [1] reported that the most difficult stage of argan production is to break dormancy in seeds. *Argania spinosa* seed exhibits mainly physiological dormancy; the dormancy caused by conditions in the embryo and endosperm may be overcome by moist-cold stratification, GA_3 and KNO_3 application [5]. Miloudi and Belkhodja [4] reported that the highest germination rate (70%)

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was determined with argan seeds soaked for 96 h and germinated at 30 °C. Soaking seeds in 1000 ppm GA_3 solution for 24 h was effective in promoting early germination [6].

This paper investigates the effects of some practical pre-sowing applications performed to increase success rate in germination of seeds for producing argan plantations under the Şanlıurfa conditions.

2. MATERIALS AND METHODS

Argan seeds used in the study were obtained from a businessman who had visited Morocco in September of 2012. Seeds were taken from a number of different argan trees, and seeds of different sizes and shapes were divided into 3 groups by their weights prior to experiment as light (<3 g), medium (3.0-4.0 g) and heavy (>4.0 g), similar to the method used by Al-Menaie *et al.* [6]. Only the seeds in medium and heavy groups were used in the study. Argan fruit is a drupe containing a stone of one, two or five nuts [7]. Therefore, among these selected seeds, argan stones with 2 strong kernels were preferred. All seeds were kept in polyvinyl bags under room conditions until the time of experiments.

From these seeds stored under room conditions, 10 stones with 2 kernels were randomly chosen for each repetition, and placed in small polyvinyl bags and closed. An experimental design was made for performing the following application, and sowing seeds in all applications at the same time. According to Alouani and Bani Aameur [5] as well as Bani-Aameur and Sipple-Michmerhuizen [3], all seeds were kept in a household refrigerator at 4 °C for 2 months until January 5th, 2013 in order to meet the resting of argan seeds.

At onset of application, seeds were soaked in 2% chlorine solution for 15 min according to Bani Aameur and Alouani [8], and 3 times quickly washed in distilled water. Stones were soaked in water at 70 °C for 5 min, except for control group [1]. The seeds in this application group were soaked in water for 24 h, 48 h, 72 h and 96 h by changing water every day in such a way to obtain the same sowing date. After soaking process in water, the stones were then scarified by slightly cracking them, and then immediately treated with the fungicide captan.

For the GA_3 application, seeds were slightly cracked and then kept in various GA_3 solutions for 24 h. The seeds taken out of GA_3 solution were washed twice with distilled water and sowed as one seed in each pot on March 10th, 2013. The seeds in control group were sowed after slightly cracking, without any further soaking treatment.

In the study, all seeds either in application or control group were sowed at 2-3 cm depth within plastic black pots of 12 cm diameter and 1 L volume filled with soil/peat and moss/sand (2/1/1). The pots were irrigated copiously at the time of planting, and later on, at optimum level. The treatment combinations were as follows:

Experiment 1. Effect of presowing soaking on germination: without pre-sowing soaking (control), 5 min dipping in hot water at 70 °C [1], pre-sowing soaking for 24, 48, 72 and 96 h in fresh water [4].

Experiment 2. Effect of gibberellic acid on germination: argan stones were soaked for 24 h in different concentrations of gibberellic acid (GA_3) solution (250, 500, 1000 or 2000 ppm) and soaked in distilled water (control) [3, 5, 6].

Percentage of germination was recorded in 2-day intervals during 38 days on the basis of cotyledon emergence. Irrigation was applied as needed. Data were statistically analyzed using randomized complete block design with two factors with three replicates (20 seeds per replicate). Analysis of variance and LSD test were used. The seed germination data were transformed using an arcsine square root transformation for normalization before statistical analysis. However, data shown in tables were not transformed. Minitab 16.1.0 Statistics software package was used for statistical analyses.

3. RESULTS AND DISCUSSION

3.1 Effect of pre-sowing soaking on germination

Pre-sowing soaking treatments and germination time had a significant effect ($p<0.001$) on seed germination of argan (Table 1). A germination rate of 10% was achieved in argan seeds sowed without soaking, while a twice higher germination rate (20.0%) was recorded in seeds kept in hot water compared to control group. In soaking applications for different periods of time, the highest germination rate was obtained with 96-h application. The study results indicated that germination rate of argan seeds soaked for 96 h increased by 100% compared to seeds soaked for 24 h (Table 2; Fig. 1).

TABLE 1 - Two-way ANOVA analysis of effects of soaking hours, days and their interactions on seed germination of *Argania spinosa.*

Source of variance	d.f.	Sum. Sq.	Mean Sq.	*F*-value	*P*-value
Soaking	5	1810.859	362.172	13.826***	<0.001
Days	5	2975.358	595.072	16.526***	<0.001
Soaking x Days	25	1741.950	69.678	1.935*	<0.05
Error	60	2160.428	36.007		

d.f.: degrees of freedom; * and ***: significant at P<0.05 and P<0.001, respectively.

TABLE 2 - Effect of pre-sowing soaking treatments on germination percentage of argan seeds.

Soaking treatments	Percent of germinated seeds on daily basis[x] Days						Total germination (%)
	14-16	18-20	22 -24	26-28	30-32	34-36	
No soaking	0.00	0.00	0.00	1.67	3.33	5.00	**10.00 d**
Hot water	0.00	0.00	5.00	5.00	5.00	5.00	**20.00 cd**
24 h soaking	0.00	0.00	5.00	6.67	10.00	6.67	**28.33 c**
48 h soaking	0.00	1.67	11.67	8.33	6.67	5.00	**33.33 bc**
72 h soaking	0.00	8.33	15.00	8.33	13.33	1.67	**46.67 ab**
96 h Soaking	1.67	8.33	16.67	15.00	11.67	3.33	**56.67 a**
Average	**0.28 c**	**3.06 b**	**8.89 a**	**7.50 a**	**8.33 a**	**4.44 b**	
	*$LSD_{0.05}$ (Soaking treatments):*9.80*; $LSD_{0.05}$ (Days):*4.00						

[x] The germination times of seeds are based on the first bloom of cotyledon leaves above pot surface. Values within rows and columns followed by different letters are significantly different by LSD test ($p<0.05$).

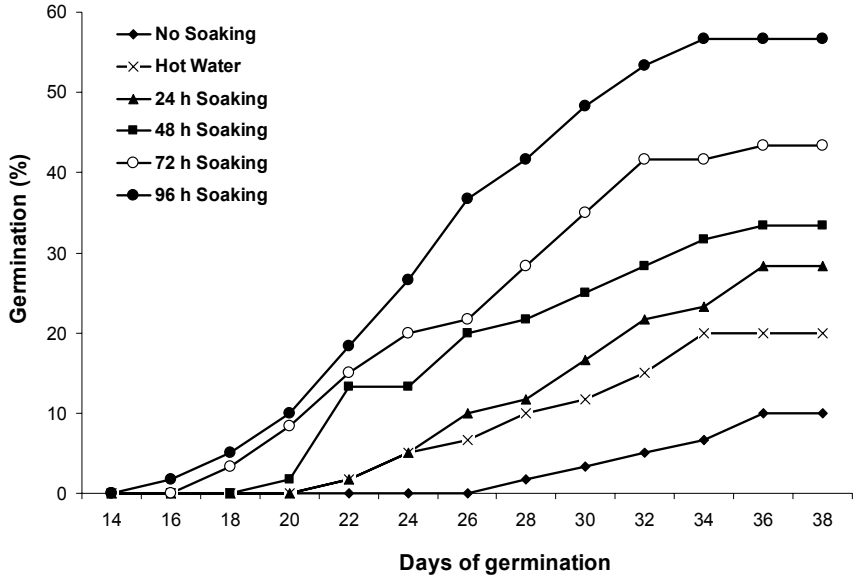

FIGURE 1 - Germination percentages of pre-soaked seeds of *Argania spinosa* in water during 24, 48, 72 and 96 h.

The first germination occurred in seeds (on the 14th day) soaked for 96 h. The highest germination rates of seeds were observed between the 22nd and 32th day, while the lowest germination percentages were observed between the 14th and 16th day (Table 2). The initial germination rate of 50% was reached on the 31st day in seeds soaked for 96 h, and this rate could not be achieved in other applications (Fig. 1)

This study is different from other studies conducted about germination of argan seeds so far. Seeds were directly sown in pot or plastic tubes similar to the studies of Alouani and Bani-Aaemeur [5] and Al-Menaie *et al.* [1].

In many other studies on germination rate of argan seeds, the seeds obtained by solid stones were directly sown in Petri dishes under laboratory conditions in which moisture, light and temperature can be controlled. Another difference of this study is that seeds were cold and dry stored at +4 ^{o}C for 2 months.

In order to break dormancy in seeds of different plant species and facilitate germination, it could be useful to apply stratification in seeds under humid conditions (sand, perlite) for different periods of time in many species. On the other hand, it could be also useful to use some other pre-sowing treatments like soaking seeds in water for different times, cold storing, keeping in hot water, wearing or cracking seed shells, keeping in GA_3, KNO_3 and thiourea [9].

There are many different factors affecting germination rate of argan seeds, including the genetic structure of the main tree from which the argan seeds were collected, annual changes in ecologies, and different seed shell thicknesses [4]. According to Çetinbaş and Koyuncu [9], in order to increase germination in fruits with hard seeds, like in *P. avium* fruits, different pre-germination treatments have been used. If the germination of the seeds is not homogeneous, researchers can use combinations of one or

more treatments with cold moist stratification to break seed dormancy.

As reported by many researchers studying the germination of argan seeds, the success rate in germination could be altered due to the genetic differences in main tree, annual rainfall, supplier country or region [4]

In the first part of this study, a high germination rate of 56.67% was achieved with argan seeds soaked for 96 h under plantation conditions. Argan seeds used in the study and showing a high germination success were initially cold-stored for 2 months, then soaked in water at 70 °C for 5 min and soaked in water for 96 h, respectively, and they were sowed after their stones were slightly cracked [3]. All the pre-sowing applications increased the germination rate of argan seeds.

Al-Manaie *et al.* [1] reported that the most difficult stage of argan production is to break dormancy in seeds. They reported that physical characteristics of seeds complicate the germination, and therefore, a germination rate of 30% would be a good result for argan seeds.

Soaking seeds for 96 h incited early germination compared to shorter soaking applications. Contrary to our results, Al-Menaie *et al.* [6] reported that long soaking application negatively affected germination due to leakage of hormones or electrolytes from seeds. However, Miloudi and Belkhodja [4] stated in their experiment of soaking seeds prior to sowing that seeds soaked for 120 h achieved 10% of germination rate on the 10th day, while this rate could be reached on the 12th day with 96-h soaking application. The researchers determined that cumulative germination rate was highest (35%) on the 23rd day (120 h soaking). Miloudi and Belkhodja [4] reported that the highest germination rate (55%) was determined with argan seeds soaked for 120 h and germinated at 25 °C, but a higher germination rate (70%) was recorded in seeds soaked for 96 h and germinated at 30 °C.

In many studies carried out on argan seeds, larger seeds were reported to show better germination. Al-Menaie *et al.* [6] reported that the highest germination rate (66%) was achieved when soaking argan seeds weighting over 4 g for 24 h; however, the small and medium-sized argan seeds showed 44% of germination. The findings of this study are similar as results of our studies. In the current study, the highest germination rate was obtained with long soaking application (4, 3 or 2 days). Nevertheless, Al-Menaie *et al.* [6] determined that directly sowed seeds showed better germination performances than other seeds soaked in fresh or warm water.

Yasin and Gübbük [10] stated that the highest germination rate (77.08%) was obtained in carob seeds with 2-h soaking at +40 °C, while the lowest germination was recorded in the control group. In the current study, argan seeds soaked in hot water for a short period of time or in mild water demonstrated higher germination rates than control group, which is similar to the results of Yasin and Gübbük [10].

3.2 The effects of pre-sowing GA_3 treatments

Pre-sowing GA_3 treatment significantly enhanced germination of argan seeds ($p<0.001$) (Table 3). In the presowing GA_3 application of argan seeds, germination rates of seeds were observed to increase in parallel with the increasing GA_3 dose. GA_3 treatments at 2000 ppm concentrations yielded the highest germination percentage (58.33%). At the end of the observation time of this study, seeds subjected to 2000 ppm GA_3 application were determined to have 8-fold higher germination rates than control group (Table 4; Fig. 2).

TABLE 3 - Two-way ANOVA analysis of effects of pre-sowing GA_3 treatments, days and their interactions on seed germination of *Argania spinosa* L.

Source of variance	d.f.	Sum. Sq.	Mean Sq.	*F*-value	*P*-value
GA_3	5	1899.002	379.800	45.264***	<0.001
Days	5	3682.792	736.558	33.703***	<0.001
GA_3 × Days	25	2470.443	98.818	4.522***	<0.001
Error	60	1311.249	21.854		

d.f.: degrees of freedom; ***: significant at P<0.001.

In this part of the study, the seeds in control group (no-soaking) soaked in pure water (0 ppm GA_3) were observed to germinate on the 22nd day, while the seeds soaked for 24 h started germination on the 24th day. However, the initial germination of argan seeds occurred on the 14th day with 2000 ppm GA_3. Among all the seeds used in the study, the germination rate higher than 50% was firstly observed on the 26th day with 2000 ppm GA_3 application (51.67%). The germination of 50% could not be reached with other GA_3 doses until the end of experiment (38th day) (Fig. 2).

According to the study results, germination rates of argan seeds increased in parallel with GA_3 doses. The earliest germination (on the 14th day) was observed in seeds treated with the highest dose (2000 ppm GA_3), and no further germination was observed in seeds subjected to GA_3 treatment after the 32nd day. Germination time of seeds decreased with higher GA_3 dose, and the highest germination rate occurred between the 20th and 22nd day (Table 4).

GA_3 has been found to be effective in increasing germination in several species, and to break dormancy in dormant seeds. Pre-treatment of blueberry seeds with GA_{4+7} (100–500 mg/L) accelerated germination [11]. The treatments of 500 and 1000 ppm of GA_3 have been successful in breaking dormancy of fig (*Ficus carica* L.) cultivar "Sarılop" seeds [12]. *Arbutus andrachne* L. (eastern strawberry tree) seeds treated with 250 mg/L GA_3 had 86% germination [13]. The combined treatment of 250 mg/L GA_3 and 100 days of stratification yielded 96% germination of black mulberry (*Morus nigra* L.*)* seeds [14]. In another similar study, a germination rate of 60.85% was achieved with the stratification of shelled stones of *Prunus avium* L. (mazzard cherry) for 120 days, and then treated with 500 ppm GA_3 [9]. It has been reported that germi-

TABLE 4 - The effect of different GA_3 concentrations on germination percentage of argan seeds.

Soaking treatments	Percent of Germinated Seeds on daily basis[x]						Total germination (%)
	12-14 days	16-18 days	20-22 days	24-26 days	28-30 days	32-34 days	
No soaking	0.00	0.00	3.33	10.00	3.33	0.00	**16.66 d**
GA_3 0 ppm	0.00	0.00	0.00	6.67	1.67	0.00	**8.34 e**
GA_3 250 ppm	0.00	0.00	8.33	8.33	5.00	6.67	**28.33 c**
GA_3 500 ppm	0.00	3.33	11.67	10.00	8.33	5.00	**38.33 b**
GA_3 1000 ppm	0.00	10.00	15.00	11.67	5.00	1.67	**43.34 b**
GA_3 2000 ppm	1.67	20.00	20.00	10.00	6.67	0.00	**58.34 a**
Average	**0.28 d**	**5.56 b**	**9.72 a**	**9.44 a**	**5.00 b**	**2.22 c**	

*$LSD_{0.05}$ (GA_3 treatments):*5.62*; $LSD_{0.05}$ (Days):*3.12

[x] The germination times of seeds are based on the first bloom of cotyledon leaves above pot surface. Values within rows and columns followed by different letters are significantly different by LSD test ($p<0.05$).

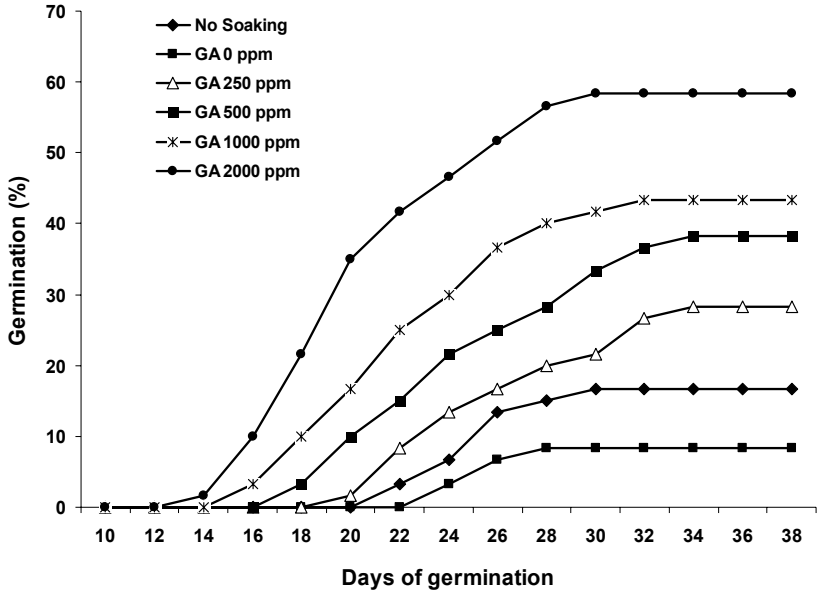

FIGURE 2 - Effects of various GA_3 treatments on seed germination percentage of *Argania spinosa*.

nation can be induced by gibberellic acid in *Vaccinium myrtillus* L. [15], *Vaccinium corymbosum* L. [16], and *Penstemon digitalis* cv. Husker Red [17] seeds. These results confirm that GA_3 treatment enhances seed germination.

Some researchers [5, 6], especially studying germination of argan seeds, reported that GA_3 treatment increases the mean germination rate and reduces the germination period. The study results are compatible with the findings obtained by Alouani and Bani Aameur [5] and Al-Menaie *et al.* [6].

In the study conducted by Al-Menaie *et al.* [6] with argan seeds sowed in Petri dishes under controlled conditions, the highest germination rate (30%) was obtained with 500 ppm GA_3 solution compared to 1000 and 2000 ppm GA_3 doses. However, in the current study, the highest germination rate (58.33%) was obtained with 2000 ppm GA_3 dose. The differences between both studies can be explained with the fact that argan seeds were subjected to different treatments herein compared to the applications used by Al-Menaie *et al.* [6], and there were genetic differences between the main trees from which the argan seeds were collected.

Alouani and Bani Aameur [5] reported that the combined application of GA_3 and cold storage (+4 ^{o}C) for 1-3 months increased the germination rate to 79.50%. The same researchers stated that seed dormancy was largely broken with two applications. Although germination was not made under controlled conditions in the current study, the applications, such as cold storage and slightly cracking stones prior to GA_3 treatment, were among the most important factors in increasing germination rate.

4. CONCLUSIONS

All these results indicate that a high germination rate can be achieved with the combined execution of cold storage of argan seeds at +4 ^{o}C for at least 2 months and soaking for at least 4 days prior to sowing or cold storage

at +4 °C for at least 2 months and treatment with 2000 ppm GA_3 for 24 h prior to sowing.

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