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Seed Germination of Argan (*Argania spinosa* L.)

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Abstract: The argan (*Argania spinosa* L.) is a multi purpose tree with good potential for growing under extreme soil and climatic conditions. In view of its potential, efforts were made to introduce this tree to Kuwait and to determine its suitability for greenery applications and oil production and, to standardize technology for mass production of superior quality seedlings. Since seeds will be imported to Kuwait and may be in short supply, studies were conducted to investigate the effect of pre-sowing soaking treatments, application of germination promoters (gibberellic acid and potassium nitrate) and dipping in hot water on seed germination and seedling establishment. Results showed that none of the pre-sowing treatments was effective in promoting germination; but it reduced the time for initiation and achieving 50% germination. Soaking in potassium nitrate gave higher germination rate than gibberellic acid. In contrast, treatment with 0.1% Benlate® to control fungal infection of seeds during the germination resulted in delayed germination.

Key words: Gibberellic acid · potassium nitrate · seed soaking · seedling development · multi purpose tree

INTRODUCTION

The argan tree (*Argania spinosa* L.), an indigenous plant of Morocco, plays a vital role in the local food chain and the environment. Natives collect argan seeds and extract oil that is valued for its nutritive, cosmetic and medicinal properties. Leaves and tender branches are being used as animal feed. Thus, it is truly a multi-purpose tree with good potential for growing under extreme soil and climatic conditions. Because of Kuwait's harsh climatic conditions and poor soil fertility and scarce water resources, only hardy and stress tolerant species can be grown successfully for agriculture production, greenery and landscape development. Since argan tree adapts well to the hostile environments and withstands irrigation with brackish water [1], has deep root system to protect the soil against soil erosion, controls desertification and creates a favorable microclimate for many fauna and flora [2], it was thought to be appropriate to introduce and explore the possibility of growing this tree for greenery and oil extraction under Kuwait's environment. In spite of importance of argan tree, information on its mass propagation and silvicultural practices are lacking.

Germination is a complex process that is controlled by several biological (species, seed viability, seed dormancy, seed size) and environmental (moisture availability, temperature, relative humidity, light intensity and duration) factors. Since plant species vary in their response to these factors, it is important to determine the optimum conditions and seed treatments for germination and seedling establishment under the prevailing climatic conditions. Therefore, studies reported here involve a number of seed treatments that were conducted to determine the optimum conditions for mass propagation of argan under Kuwait's environmental conditions.

MATERIALS AND METHODS

The argan seeds for the present study were collected from natural plantations around Agadir, Morocco.

Experiment 1. Effects of seed size and presowing soaking on germination: This experiment consisted of a combination of seed sizes (light (<3 g), medium (3.0 – 4.0 g) or heavy (> 4 g) and presowing soaking (24 hr soaking in fresh water, 5 min dipping in hot water at 70°C or no soaking control). Following treatment, seeds were sown in transparent polyethylene containers. The bottom of these containers was lined with tissue paper moistened with distilled water. Five seeds were incubated in each container and were covered with a thin layer of moistened absorbent cotton to maintain adequate moisture during germination. Each treatment was replicated 10 times. The seeds were observed daily to

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record germination and also to ensure the availability of sufficient moisture levels in the containers. The final germination percentage was calculated when seeds ceased to germinate for eight days.

Experiment 2. Effects of germination promoters: One hundred healthy seeds were soaked in different concentrations of gibberellic acid (0.1, 0.25, 0.5, 1.0 or 2.0g/l) or potassium nitrate (2.5 or 5.0g/l) for 24 hrs in glass beakers that were covered with black polythene sheets to avoid photo oxidation of gibberellic acid. The germination process in this experiment was conducted in the same manner as experiment 1; however, 10 seeds were sown instead of five in each container. After sowing, the containers were placed in laboratory at 25°C. Each treatment was replicated 10 times in a completely randomized design.

Experiment 3. Effects of soaking and surface treatment with fungicide on germination: Seed treatments included 5 minutes dip in hot water at 70°C, soaking in KNO_3 solution (0.5, 1.0 or 1.5 %) and no soaking (control). Treated seeds were then dipped in 1g/l solution of Benlate® (Methyl 1-(Butylcarbamoyl)-2-Benzimidazolecarbamate) before sowing. Ten seeds were sown in each container and the experiment was replicated ten times in a Randomized Complete Block Design.

Recording of observation and statistical analysis of data: Observations on seed germination were recorded daily and the total germination percentage was calculated for each treatment. The data were statistically analyzed using the ANOVA procedure [3]. Significant treatment means were separated by Duncan's Multiple Range Test.

RESULTS

Experiment 1. Effects of seed size and presowing soaking on germination: The highest germination percentage (66%) was recorded in heavy seeds with an average seed weight of >4 g (Table 1; Plate 1). In contrast, 44% germination was observed in light and medium seeds without soaking. The light and heavy seeds did not germinate when they were dipped in hot water, whereas the medium seeds showed 4% germination. Fresh water treatment also affected germination as evident by the fact that only 15 to 30% germination was noticed in the different categories of seeds.

Experiment 2. Effects of germination promoters: Soaking in water, gibberellic acid or in potassium nitrate solution affected total germination in this species (Table 2). Among the different GA_3 treatments, soaking seeds in 500 ppm solution resulted in highest germination (30%) and was followed by 100 ppm and 250 ppm. Increasing the

Plate 1: Germination of argan seeds in different soaking treatments
NS = No Soaking FWS = Fresh Water Soaking HWS = Hot Water Soaking

Table 1: Effects of Size and Pre-sowing Treatments on Germination of Argan Seeds (±SE)

Seed Size[x]	Seed Treatment[y]	No. of Germinated Seeds on Daily[z] 13	15	21	24	Total	Final Germination (%)
Light	Fresh Water	7	2	2	1	12	24
	Hot Water	--	--	--	--	0	0
	No Soaking	20	--	2	--	22	44
Medium	Fresh Water	6	3	5	1	15	30
	Hot Water	1	--	1	2	4	8
	No Soaking	20	2	--	--	22	44
Heavy	Fresh Water	6	1	1	--	8	16
	Hot Water	--	--	--	--	0	0
	Seeds No Soaking	32		1	--	33	66
Significance[t]	Seed Weight					NS	NS
	Soaking					** (±0.40)	** (±4.03)
	Seed Weight X Soaking			* (±0.19)	* (±1.87)		

[x]Seed Sizes used - Light seeds = < 1.5 g; Medium Seeds = 1.5 – 3.0 g; Heavy seeds = >3.0 g.

[y] Seeds were soaked in fresh water for 24 hrs or dipped in hot water at 70°C for 5 min.

[z]Days after sowing. Total number of seeds used for each treatment was 50 (10 replications each of 5 seeds).

[t]Data were analyzed using one-way ANOVA procedures at P <0.05. Std. errors of mean values are given in the parenthesis.

Table 2: Comparison of Various Gibberellic Acid and Potassium Nitrate Treatments on Germination of Argan Seeds

Treatments[*]	Number of Germinated Seeds on Daily[**] 4D	5D	6D	8D	11D	13D.	14D.	15D.	18D.	19D.	Total
No Soaking		8	12	30	12		2				64 d
Fresh Water	2	9	2	7	2		3	3		1	29 b
GA100 ppm		9	1	7			6		2	1	27 b
GA250 ppm		12	6	1	1		1	4		1	26 b
GA500 ppm	1	6	5	2	5	1	2	4	2	2	30 bc
GA1000 ppm		4	4	1			3	1	1		10 a
GA2000 ppm		4	2	2	2		3	3			16 ab
0.25% KNO_3	3	13	11	3	8		3	3		1	43 c
0.5% KNO_3	3	14	13	7	4		1	4			48 c
Std. Error of Mean[***]										±6.7	

[*] Seeds were soaked in fresh water, gibberellic acid or potassium nitrate solutions for 24 hrs prior or sowing; Seeds with 1 mm or longer plumule were considered as germinated.

[**] D = Days after sowing. Total number of seeds used for each treatment was 100 (10 replications each of 10 seeds).

[***] Data were analyzed using one-way ANOVA procedures. Significant means were separated by Duncan's Multiple Range Test. The means followed by the same alphabet are not significantly different at P <0.05.

Table 3: Effects of KNO_3 and Hot Water Treatments on Seed Germination of Argan (± S.E)

Treatments[*]	Number of Germinated Seeds on Day 6	8	11	13	15	20	22	25	26	32	Total
Control	2	22	34	4	2	5					69
Hot Water		4	3	1	1	2		2	1	3	17
0.5% KNO_3	12	23	10	4	1	2				1	53
1% KNO_3	12	10	6	2	5	2	3			1	41
1.5% KNO_3	10	10	17	2	1	4	1			1	46
Significance at P < 0.05										**	
Std. Error of Mean[***]									±7.8		

[*] Seeds were soaked in potassium nitrate solutions for 24 hrs prior or sowing; Seeds with 1 mm or longer plumule were considered as germinated.

[**] Total number of seeds used for each treatment was 100 (10 replications each of 10 seeds).

[***] Data were analyzed using one-way ANOVA procedures. * denotes significance different at P <0.05; ** denotes significance at P <0.01. Means were separated by Duncan's Multiple Range Test. The means followed by the same alphabet are not significantly different at P <0.05.

concentration further did not have any effect. Similarly, soaking in KNO_3 resulted in 43-48% germination, which was lower than that in control, but higher than in GA_3. However, soaking in water, gibberellic acid and KNO_3 induced early germination. In case of GA_3 and KNO_3, maximum germination was observed five days after soaking. In contrast, control seeds (no soaking) showed the highest germination (64%), but maximum germination was observed on the eighth day (Table 2).

Experiment 3. Effects of soaking and surface treatment with fungicide on germination: Fungicide treatment delayed the germination by nearly a week, but the total germination percentage was not affected (Table 3). Moreover, the fungal growth was effectively controlled. The maximum germination (69%) without dipping in Benlate® was observed in the control treatment (Table 3). This was followed by 0.5% KNO_3 (52%), 1.0 % KNO_3 (41%) and 1.5 % KNO_3 (46%). KNO_3 treatment promoted early germination as well as reduced the time required for 50% germination. In contrast, dipping seeds in hot water reduced the total germination from 69% to 17%.

DISCUSSION AND CONCLUSIONS

The control treatment of seeds in this species showed 44 to 69% germination which indicates that a number of seeds were either dormant or nonviable at the start of the experiment. A number of treatments have shown improvement in germination of dormant seeds. Interestingly, soaking in water or pretreatment with GA_3 or potassium nitrate, although, did not improve total germination, it reduced the time for initiation of germination and time required to achieve 50% germination. This showed that these treatments were effective in inducing metabolic activity in the embryo required for the initiation of germination process. However, prolonged soaking in water or chemical solution may have caused the leaching of naturally occurring hormone or electrolyte from the seed which adversely affected the germination. Therefore, it may be worthwhile to assess the effect of reduced soaking period on germination. In contrast, hot water treatment was detrimental to germination thereby eluding to the fact that hot water probably damaged the embryo that led to germination failure. Further studies, however, are required to understand the actual mechanism involved in the initiation of germination process in this species.

Experiments showed that argan seeds without soaking prior to sowing germinated better than those soaked in fresh water or dipped in hot water. Soaking seeds in fresh water or growth promoters like gibberellic acid or potassium nitrate solution although affected total germination percentage, it reduced the time required for initiation of germination and time required for 50% germination. Treatment of seeds with 0.1% Benlate® prior to sowing effectively controlled fungal infection, but delayed the germination slightly.

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