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Effect of Different Pretreated Methods on Seedling Development of El-Gabal El-Akhdar Carob Tree (*Ceratonia siliqua*)

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ABSTRACT

Carob tree (*Ceratonia siliqua* L.), is a small to medium sized broadleaf, a slowly growing, woody evergreen, sclerophyll, and widespread specie occurring as a native plant in the Mediterranean Basin. Carob tree is an environmentally and economically important tree and it is an evergreen native wild species found naturally in El-Gabal El-Akhdar region which is located in the coastal belt of the northeastern region of Libya. Seeds pretreated with different methods were allowed to continue their growth and the measurements of development were studied. Carob seedlings which produced from pretreated with soaking in boiling water showed significant differences in their fresh weight, root and shoot lengths compared with untreated control seedlings, while results of dry weight was not significant compared to control treatment. The effect of using sulfuric acid pretreatments with different exposure periods, showed no significant differences in seedling parameters (dry weight, fresh weight, shoot and root lengths) compared to control. Because of its good ability to germinate and develop after treatment with boiling water which is easy, cheap and available, the study recommended that the Libyan carob can be used in any restoration, rehabilitation and reforestation programs.

تأثير معاملات مسبقة مختلفة علي تطور بادرات نبات خروب
الجبل الأخضر *Ceratonia silique* L.

سالم الشطشاط وفاطمة بورزيزة

الخروب شجرة متوسطة الحجم ذات اوراق عريضة بطيئة النمو خشبية دائمة الخضرة تنتشر بشكل كبير كنبات محلي في منطقه حوض البحر المتوسط ويعتبر الخروب احد الاشجار المهمة من النواحي البيئية والاقتصادية وهو ينمو بشكل بري وطبيعي في منطقة الجبل الاخضر التي تقع في الجزء الشرقي الشمالي من ليبيا على طول الساحل. في هذه التجربة تركت البذور التي عوملت مسبقا بطرق مختلفة لتنمو ثم قيس بعد ذلك مجموعة من المتغيرات المتعلقه بتطور البادرة وقد بينت النتائج ان البذور التي سبق معاملتها بالماء الدافئ اعطت اختلافات معنوية في كل من الوزن الطري وطول الجذر والساق مقارنة بالبذور الغير معاملة في حين لم تكن نتائج الوزن الجاف ذات معنوية عند مقارنتها بالمشاهدة. كما بينت الدراسة أن استخدام حمض الكبريتيك علي فترات زمنية مختلفة لم يظهر اي اختلافات معنوية في كل قياسات تطور البادرة مقارنة بالغير معاملة. وبالنظر الى قدرة نبات الخروب على الانبات والتطور بعد المعاملة بالماء الدافي والتي تعتبر سهلة ورخيصة ومتاحة فان هذه الدراسة توصي باستخدامها كما توصي باستخدام نبات الخروب في برامج الاعاده البيئية او التاهيل البيئي وكذلك برامج زراعة الغابات.

INTRODUCTION

Carob tree (*Ceratonia siliqua* L.) is a broadleaf evergreen species occurring as native plant in the Mediterranean Basin (Ramón-Laca and Mabberley, 2004). With its ability to develop different strategies for adapting to water stress (Catarino, 1993), which is the most character resulting of the climatic conditions. This explaining its large distribution in the arid and semi-arid Mediterranean climate (Correia and Martins-Loucao, 1994; Lo Gullo and Salleo, 1988).

It is a component of the Mediterranean vegetation and is well known in the Mediterranean countries for its ornamental, nutritional, and medicinal value (Batista et al., 1996; Battle and Tous, 1997). The combination of human activity and the Mediterranean climate typically results in fast soil erosion and plant cover deterioration. Using pioneering species like the carob tree in reforestation initiatives and restoration of degraded soils remains an effective technique to accomplish avoidance or management like these consequences (Rejeb, 1995).

In Libya, Carob considered as one of the most important species of the plant vegetation cover of El-Gabal El-Akhdar region and form mixed woodland together with Juniperus phoenicea, Olea europaea, Pistacia lentiscus, Quercus coccifera, Cupressuss empervirens and Pinus halepensis. El-Gabal El-Akhdar is located 881 meters above sea level and stretches for approximately 300 kilometers along the coast belt. The region has a Mediterranean climate, 250–600 mm of annual rainfall on average, and terrarossa or heavy clay soils (Johnson, 1973; Sharaf, 1971; El-Zwaam 1995).

Like other legume plants, carob seeds exhibit seed coat impermeability to water. Therefore, the seed requires seed coat scarification in order to mature (Piotto and Piccini, 1996; Piotto and Ciccarese, 1999; Piotto and Di Noi, 2003). According to Batlle and Tous (1997), Güneş et al. (2009), and Güneş et al. (2013), seeds can be treated with tap water, boiling water, sulfuric acid (**H_2SO_4**), or gibberellic acid (**GA_3**) to increase germination. The development of the seedlings is improved by these treatments. In these cases, the dormancy-breaking therapies utilized need to increase gas exchange and moisture absorption while avoiding changes to the embryo and endosperm (Baskin & Baskin, 1998).

This study was aimed to evaluate and test the seedling development and monitoring the possibility of development of *Ceratonia siliqu* under local Libyan environmental conditions and provide the authorities with the data to propagate or to use this plant in El-Gabal El-Akhdar area in a replacement process of some threatened plants, prevention of soil erosion, and restoration programs. In addition, to use it in gardening and forests development programs.

MATERIALS AND METHODS

Plant material:

The plant materials (pods) were collected from different locations at El-Jabal El-Akhdar on a different height above sea level and seeds were extracted from the pods manually. Seeds were treated with four different methods (Elshatshat and Borziza 2023). Briefly; soaking in the tap water, boiling Water (100 C˚), scarification with (**H_2SO_4**) for 5, 10 and 15 minutes, mechanical scarification using rough paper and the last one is control (untreated seeds). 5 seeds of each treatment were germinated using Petri dich with moisten Whattman filter paper and Petri dishes were randomized in a precision incubator and maintained in the dark at 22±0.5C°. Germinated seeds of carob were allowed to develop under the same conditions. The seedlings were daily monitored and the growth parameters were measured (Figure 1).

Fig. (1): Measurements of seedling development of Carob plant.

Measurements of seedling developing:

At the end of the growth period in this study, root length, shoot length, fresh and dry weight of the grown plant were measured. Fresh weight was measured directly by sensitive balance while dry weight was taken after drying of the plant in an oven at 65C° for 24 hours. The seedling vigor index was calculated according to the formulae as following:

SVI = (Shoot length+ Root length) × Germination percentage.

Statistical Analysis:

Variables were displayed as means and standard deviation. The study was analysed by analysis of variance (ANOVA) using SPSS (version 26) to find out the statistical differences in the means of seedlings parameters. parameters were collected individually and the mean of the parameters were calculated and compared with the control using independent T test.

RESULTS AND DISCUSSION

Seedling development of boiling water pre-sowing treatment:

In this treatment the means the fresh weights of seeds treated with boiling water and control were significantly different (p- value < 0.05). While, the mean of the dry weights was not significant (p- value > 0.05). The differences in the mean of the root and shoot lengths of the treated seedlings and control was highly significant (p- value < 0.05) (Table 1).

Table (1): Evaluation of seedling parameters in boiling water.

Group Statistics				I. S. Test	
Parameter		N	Mean ± STD	T	Sig.
Fresh weight	Treatment	24	0.43±0.08	7.793	0.00
	Control	8	0.18±0.05		
Dry weight	Treatment	24	0.14±0.16	1.405	0.17
	Control	8	0.066±0.043		
Root length	Treatment	24	4.18±0.86	6.597	0.00
	Control	8	2.07±0.45		
Shoot length	Treatment	24	3.45±0.59	4.396	0.00
	Control	8	2.33±0.72		

Seedling development of mechanical scarification pre-sowing treatment:

In this treatment the means of the fresh weights of seedlings pre-treated with mechanical scarification and control were significantly different (p- value < 0.05). Both the means of the seedling dry weights and control were also significant (p- value < 0.05), and the means of the root length of the seedling and control were also significant (p- value < 0.05), while the means of the shoot length of the seedling and control were not significant (p- value > 0.05), as shown in the Table (2).

Table (2): the effect of mechanical scarification on seedling growth parameters.

Group Statistics				Independent Samples Test	
Parameters		N	Mean ± STD	T	Sig.
Fresh weight	Treatment	10	0.41±0.08	3.698	0.004
	Control	3	0.21±0.07		
Dry weight	Treatment	10	0.15 ±0.04	3.513	0.005
	Control	3	0.04 ±0.04		
Root length	Treatment	10	3.76±0.7	2.609	0.024
	Control	3	2.57±0.5		
Shoot length	Treatment	10	3.55±0.88	0.968	0.354
	Control	3	3.03±0.4		

Seedling development of Sulfuric acid pre-sowing treatment:

As shown in the Table (3), the results showed that the means of the fresh weights of seedlings treated with sulfuric acid for 5 minutes and control were significantly different (p- value < 0.05). the means of the seed dry weights and control were not significant (p- value > 0.05), also the means of the root and shoot length of the seedling compared with control were also not significant (p- value > 0.05).

On the other hand, the results revealed that seedlings pre-treated with sulfuric acid for10 minutes and control were significantly different (p- value < 0.05). but the means of the seedling dry weights and control were not significant (p- value > 0.05), also the means of the root and shoot length of the seedling compared with control were not significant (p- value > 0.05) as shown in the Table (3). When pre-treatment with sulfuric acid for 15 minutes compared with control reflected non-significant change (p- values > 0.05), which means comparable results were found for each parameter compared with control (Table 3).

Comparisons using one-way test of variance to determine the differences in the means of seedling parameters in all pre-sowing treatments, showed no significant differences in the mean of fresh and dry weight, root and shoot lengths among all pre-sowing treatments (p- value > 0.05).

The results showed that carob seeds had different vigor indexes in different pre-treatments, which was higher in the seeds treated with boiling water, followed by seeds treated in sulfuric acid for 15 minutes and similar results were found in the seeds treated with mechanical scarification and sulfuric acid for 5 minutes. The lower seedling vigor index was recorded by seeds treated with sulfuric acid for 5 minutes.

Carob (*Ceratonia siliqua* L.) is one of the most environmentally and economically an important tree with slow growing character and it can reach around 100 years from its life period and begins fruiting after 6 or 7 years of growth. As a result, there is a greater need for an increase in production volume of Carob plants. The carob tree is more difficult to grow well than is often believed when using horticulture techniques to get such outcomes. Seed dormancy, which is caused by the seed coat's apparent impermeability to water, is another challenge to be overcome. Furthermore, carob tree seeds undergo a period of physiological dormancy that is contingent upon the environmental circumstances in which the tree is found (Gruendel et al., 2006).

		Exposure time of H_2SO_4								
		5min.			10min.			15min.		
Parameters		Mean ± STD	T	Sig.	Mean ± STD	T	Sig.	Mean ± STD	T	Sig.
Fresh weight	Treatment	0.42±0.065	-2.125	0.06	0.34±0.07	-2.228	0.042	0.43±0.089	-2.054	0.052
	Control	0.49±0.025			0.43±0.09			0.50±0.051		
Dry weight	Treatment	0.15±0.035	-0.728	0.483	0.14±0.027	-0.958	0.353	0.16±0.036	-1.524	0.143
	Control	0.17±0.016			0.15±0.033			0.18±0.013		
Root length	Treatment	3.63±0.87	0.428	0.678	3.53±0.87	0.409	0.688	4.36±0.73	0.693	0.495
	Control	3.4±0.8			3.33±1.17			4.14±0.79		
Shoot length	Treatment	3.86±0.72	1.144	0.279	3.73±1.03	-0.39	0.702	3.66±0.77	-0.613	0.546
	Control	3.35±0.76			3.96±1.37			3.89±1.06		

Table (3): Seedling characters for different periods of H_2SO_4 in minutes using independent samples test.

Since carob seeds have an impermeable and strong seed coat that prevents water absorption, APAT (2003) found that the average germination of carob seeds was 60–95% following treatment. Naturally, this has an impact on the growth of seedlings. Treatments include scarification, mechanical application of corrosive substance, soaking in hot water for 12 to 24 hours, application of acid (such as H_2SO_4), and the enhancement of seed germination by certain alkalis. According to our earlier research on the germination of Carob plant seeds, all pre-treatments improved seed germination to varied degrees.

The germinated seeds in each treatment were underwent different measurements for their seedlings fresh and dry weight, root and shoot lengths were also measured these parameters were collected individually and the mean of the parameters were calculated and compared with the control using independent T test. pre-treated with soaking in boiling water showed significant differences in their fresh weight, root and shoot lengths compared with untreated control seeds while results of dry weight was not significant compared to control treatment. in previous study by (Saif, 2020) demonstrated that significantly highest root length was obtained with the hotbed treatment compared to untreated seeds resulted in the highest percentages in seedlings growth parameters.

According to Baskin and Baskin (2014), high temperatures during the germination process can promote seed germination by breaking physical dormancy. Furthermore, Kozlowski and Pallardy (1997) noted that in certain cases, heat higher than 50 degrees Celsius was necessary to break the dormancy of certain leguminous plant seeds. In mechanical scarification pre-treatment the means of fresh and dry weights in addition to root length was highly significant compared to untreated control treatment, while shoot length showed no significant differences compared to control. The scarification treatment improved morphological measurements of carob seed.

All sulfuric acid pre-treatments showed no significant differences in seedling parameters (dry weight, fresh weight, shoot and root lengths) compared to control.

Comparison between these pre-treatments performed, the greatest fresh weight was obtained by seeds pre-treated with boiling water and sulfuric acid for 15 minutes, the greatest dry weight and root length were obtained by pre-treatment with sulfuric acid for 15 minutes, but the maximum shoot length was obtained by soaking in sulfuric acid for 5 minutes. generally, no significant differences in the mean of each individual parameter in each pre-treatment, so dry weights, fresh weigh, shoot and root lengths showed no significant differences in each pre-treatments.

El Deen et al, (2014) studied carob seed propagation and their findings illustrated that the fastest germination, the greatest plant length, number of leaves/plant, root length and dry weight were acquired by soaking seeds in 60% $\mathbf{H_2SO_4}$. According to Tsakaldimi and Ganatsas (2001), the hot water treatment resulted in the shoot and root lengths because it damaged the embryo. Gunes et al., (2013) reported the maximum root length found in sulfuric acid treatment and followed by mechanical scarification of the seeds. However, the greatest shoot length was determined with mechanical scarification and the lowest hot water treatment (Gunes et al., 2013).

As far as it is known in seed germination, when seeds are placed in an environment that is moist and has oxygen, they begin to absorb water, break down the food that has been set aside, start to convert into useful metabolites, and germinate. Water enters the seed through its seed coat, however no germination may take place if the seed coat is impermeable to water because of physiological or natural dormancy. This is a typical plant life cycle phenomenon that helps the species survive or adapt to harsh environmental conditions. In like this case, different techniques were used to soften the seed coat and subsequently, resulted in germination, which sped up the growth of seedlings. The hard seed coat that prevents water from penetrating is largely responsible for the seed dormancy of Fabaceae species, of which carob is a part. But in this instance, treating the seeds was necessary to improve germination when they were planted for these objectives.

CONCLUSION

It is clear from this study that using pre- boiling water gave a very good results in development of carob seedlings while it caused very high seed germination, therefore, it can be classified as the best pre-treated method to produce Carob seedlings. Carob serves as an ornamental and roadside tree and as a fence against sand and some storms. Thus, using it in rehabilitation or restoration programs at El-Gabal El-Akhdar area must take in account. While it can be used in a replacement of some threatened species, it can be also utilizing to prevent, control or low the effect of erosion in the area, especially, after Daniel storm that strike the north eastern part of Libya in September 2023.

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