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Original Article

Capability of Wild Carob Seeds in Benghazi City, Libya to Germinate and Establish

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Abstract
This study investigated the germination and growth potential of carob (*Ceratonia siliqua* L.) seeds collected from three sites in Benghazi City, Libya, under various water regimes and environmental conditions. As a drought-resistant species critical for sustainable agriculture in arid regions, carob faces challenges such as reduced cultivation areas resulting from climate change and poor management. This research evaluated pod and seed attributes at three locations: Quarsha, Sidi Ali, and Tika, and found significant differences among them in pod length, pod weight, number of seeds per pod, and seed weight, with Tika producing the most promising results. Germination tests demonstrated that mechanical scarification significantly enhanced seed germination, while moderate water levels (8–10 ml) proved ideal for seedling growth, particularly in terms of shoot and root development. The study also emphasises the vulnerability of carob seedlings to water stress, noting that root growth was more adversely affected than shoot growth. It can be concluded that site selection and effective water management are crucial to carob growth and establishment success. The study indicates that carob is a tolerant crop species in the extremely arid climate of Libya, advocating for improved irrigation systems, seed treatment methods, and the conservation of genetic resources for sustainable agriculture. Further field trials are suggested to confirm these results in natural settings.
Keywords: Carob, *Ceratonia Siliqua*, Libya, Seed Germination, Early Establishment.

Introduction

Carob (*Ceratonia siliqua* L.) belongs to the Caesalpiniaceae family [1]. It thrives in warm temperate and subtropical areas with cool winters, pleasant to warm springs, and hot, dry summers. There remains some uncertainty regarding the natural distribution of Carob in the Mediterranean. It was believed to have originated in the eastern Mediterranean- likely in Syria, Turkey, and possibly Yemen [2]. However, recent findings suggest a possibility of its presence in the High Atlas region of Morocco during the late interglacial period, subsequently migrating north and eastward [3].

The carob tree is evergreen and can reach up to 15 meters in height. Its leaves are paripinnate, measuring up to 30 cm long, producing unisexual flowers. The pods measure approximately 7.5 to 20 by 1.2 to 2.5 cm and are compressed, thick, turgid, and curved. The seeds are pale brown and shiny within the pulp. Carob thrives in various soils, including poor sandy types and rocky hillsides with depths of 20 to 30 centimeters. However, limited water storage can restrict both growth and yield [4,5].

Optimal growth occurs in sandy, well-drained loams that are moderately rich, containing low to medium organic matter and a pH between 5.7 and 6.6. Calcareous soils with elevated lime content, reaching at least a pH of 7.9, are also adequate [1,4-8]. In Cyprus, it primarily grows on calcareous soils, which can be reddish, featuring around 20% calcium carbonate over a highly calcareous subsoil, or white and highly calcareous, containing up to 80% calcium carbonate [5]. Both soil types overlay limestone bedrock.

Although Carob grows while exposed to full sunlight, it was found that the leaves of carob seedlings grown in 10% sunlight were half the thickness of those exposed to full sunlight [9]. Mean fruit mass ranges from 4 to 29 g across its distribution, with an average mass of 11–18 g in the Algarve [1,10,11], peaking at a mean of 38 g in Sicily [12]. The mean fruit mass shows a similar range for both wild and cultivated trees. In Morocco, smaller fruits are associated with drier conditions [13]. Despite the variation in fruit size, the number of seeds per fruit is less variable, ranging from 5 to 14 in the Algarve [10], with an average between 8 and 17 [12,14-19]. By weight, carob fruits consist of 73%–95% pulp and 8%–16% seeds, varying among cultivars [1,20].

Many studies have shown that the mean seed mass of Carob varies from 0.14 to 2.44 g [10,14,16,19,21]. This variation partly depends on climate, with the largest seeds found in semi-arid lands and the smallest in subhumid and arid regions [13,16,22]. Additionally, seed size tends to increase with altitude and latitude [22]. In Libya, Carob is considered one of the most important crops due to its many advantages, including its drought resistance and ability to grow in poor soil. However, some challenges are encountered in Carob cultivation in Libya, which, in turn, affect its spread and cultivation [2].

There is an apparent decline in areas planted with Carob. In recent years, several factors have caused the decrease in areas dedicated to carob planting, including a lack of care and management, such as the absence of expansion programs in suitable locations and the lack of updated farming techniques [2]. Agricultural crops are significantly affected by drought and climate change in various parts of Libya. Conflicts have also ravaged agricultural zones or converted them to non-agricultural purposes. Economic challenges compel farmers to choose more lucrative and easier crops over carob [2]. Nevertheless, initiatives are in progress to

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improve carob cultivation due to its numerous advantages, including its applications in the food and medicinal fields. By the end of the last century, labour costs rose, which decreased carob production profitability, prompting farmers to switch to more profitable crops [23,24]. Furthermore, semi-natural populations are under threat by agricultural and urban expansion in coastal regions [25], coupled with a decline in traditional usage.
Large areas of carob were removed due to extreme weather conditions and the resulting devastating disasters, such as those caused along the coast of Libya by Storm Daniel in September 2023. As a result of this decline, carob has been included in national lists of priority forest genetic resources for conservation and management in several countries, including Tunisia and Lebanon [25,26]. Regarding adaptation to climatic conditions, carob is a type of tree that grows in arid and marginal environments, so understanding its developmental effects is essential for determining the best methods to ensure the trees and similar species survive even in challenging climatic conditions. Moreover, studying the impact of irrigation systems on carob can help select efficient irrigation methods that use less water, thus contributing to the conservation of water resources in areas facing water scarcity.
Carob plays a vital role in sustainable agriculture; understanding its behaviour under different irrigation systems can help improve agricultural techniques and reduce negative environmental impacts. This study aids in developing sustainable agricultural strategies that support the local economy, particularly in regions reliant on agriculture under challenging environmental conditions. It also enhances our understanding of how to adapt to the challenges of current and future ecological situations. This study aims to evaluate the productivity of carob trees in the vicinity of Benghazi city in eastern Libya, focusing on seed quantity, pod size, seed germination rates from various locations in the area, and the ability of carob seedlings to grow and develop under different watering regimes.

Methods

Area of study

Three sites were selected around Benghazi based on the availability of wild carob trees. The three sites were as follows: Tika, located near the western entrance to the town, at the coordinates 31°55'52.37" N and 20°00'46.20" E. The second site was Quarsha, situated in the western part of the city, at the coordinates 32°02'00.15" N and 20°04'11.87" E; and the third site, Sidi Ali, was positioned at the eastern entrance of the town, at the coordinates 32°19'30.29" N and 20°16'18.29" E.

Pod and seed collection, and germination experiment

Two hundred pods were collected randomly from trees at each site around the city. The length of the pod was measured with a string and a ruler, while the number of seeds, their weight, and the weight of the pods were measured on an analytical balance. Seed dormancy was then broken by mechanical scarification with sandpaper. Ten scarified seeds were placed in each Petri dish under complete sterile conditions. The seeds in the dishes were watered with varying amounts of water at specified intervals (2, 4, 6, 8, and 10 ml every 2, 5, and 7 days). After germination, the shoot and root systems were harvested, and their lengths were measured with a ruler. Their fresh weights were recorded using an analytical balance, while their dry weights were measured in a drying oven at 40°c for 24 hours. This experiment was conducted under sterile conditions using 70% alcohol.

Seedling performance experiment

This experiment was conducted under sterile conditions using 70% alcohol. The length of the pod was measured using a string and a ruler, while the number of seeds, their weight, and the weight of the pod were measured on an analytical balance. Seed dormancy was subsequently broken by mechanical scarification using sandpaper. The empty pots were weighed and then filled with peat moss, and their field capacity was determined. Next, 10 scarified seeds were placed in each pot using tweezers to prevent any contamination.
The seeds in the pots were watered with varying amounts of water at specified intervals. After germination, the shoot and root systems were harvested, and their lengths were measured using a ruler. Their fresh weights were taken with an analytical balance, while their dry weights were obtained using a drying oven. All readings were recorded and documented in tables.

Results

Pod and seed description

The pod length was significantly different between sites, as a one-way ANOVA test revealed that the Tika and Sidi Ali sites had longer pods than the Quarsha site, with an average length of 15.60±2.542 cm across all sites (Table 1). The same test results indicated significant differences in pod mass across the three sites. The Tika site had the highest pod mass, with 12.90±2.742 gm, followed by Quarsha and Sidi Ali (Table 1). Another one-way ANOVA confirmed that the number of seeds per pod varies significantly across the three sites. Tika has the highest number of seeds per pod with 14.14±2.036 seeds/pod, substantially greater than

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Quarsha and Sidi Ali. However, Quarsha and Sidi Ali were not substantially different from each other (Table 1). Results from the one-way ANOVA confirmed that pod seed mass in this study varies significantly across the three sites. Tika and Quarsha had similar pod seed masses of 2.32±0.438 and 2.28±0.583 g, respectively, while the Sidi Ali site had a significantly lower pod seed mass (Table 1).
The results of the One-Way ANOVA test indicated that the site factor significantly affected seed mass (100 seed mass) ($p < 0.001$). Sidi Ali had a considerably lower mean seed mass than Quarsha and Tika. Still, there was no significant difference in seed mass between Quarsha and Tika (Fig. 1). This analysis suggests that environmental or other factors at Sidi Ali may influence seed mass differently than the other two sites (Fig. 1).

Table 1. Descriptive of pod length, pod Mass, number of seeds/pod, and pod seed mass among and between sites. Different letters showed significant differences. Mean±StDev.

Variable	Site	Mean
Pod Length (cm)	Quarsha	14.33±2.206[a]
	Sidi Ali	16.24±3.065[b]
	Tika	16.23±1.691[b]
	All sites	15.60±2.542
Pod Mass (g)	Quarsha	11.27±3.167[a]
	Sidi Ali	9.90±3.159[b]
	Tika	12.90±2.742[c]
	All sites	11.36±3.256
Number of Seeds per Pod	Quarsha	12.58±2.474[a]
	Sidi Ali	11.86±3.009[a]
	Tika	14.14±2.036[b]
	All sites	12.86±2.701
Pod Seed Mass (g)	Quarsha	2.28±0.583
	Sidi Ali	1.92±0.495
	Tika	2.32±0.438
	All sites	2.17±0.539

Germination experiment
Time has a strong, significant effect on germination in Petri dishes. The number of new seedlings is highest on the first two days and decreases to five and seven days as time progresses. Water has a negligible but still significant effect. The optimal water level for germination appears to be around 10 ml (baseline), as lower water levels (e.g.,4 ml) significantly reduce seedling counts (Fig. 2).

Young seedling performance
The statistical output is from a general linear model (GLM) analyzing the interaction effects of Time (days) and Water (ml) on shoot length (mm) (Fig. 3). Time significantly affects shoot length, with 5 days showing a significant decrease compared to the baseline (7 days). However, 2 days does not differ significantly from the baseline. Water has a strong effect on shoot length. Lower water levels (2 ml) significantly reduce it, while higher water levels (8 ml) significantly increase it. Intermediate water levels (4 ml, 6 ml) do not differ significantly from the baseline (10 ml). The model explains only 16.01% of the variation in shoot length, indicating that other factors not included in the model may have a substantial influence. For longer shoot lengths, higher water levels (e.g., 8 ml or 10 ml) are recommended. Lower water levels (e.g., 2 ml) should be avoided. Time Sensitivity: Shoot length is significantly shorter at 5 days compared to 7 days, suggesting that growth may slow down or plateau after 5 days. Shoot length is susceptible to water levels, with 8 ml being particularly beneficial.

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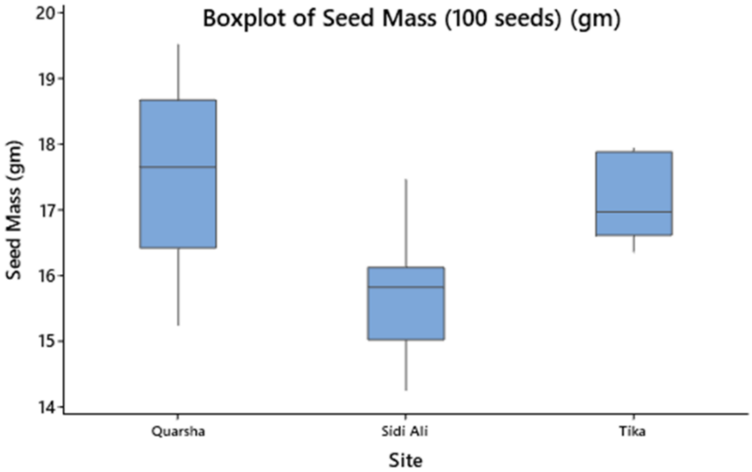

Figure 1. Seed mass,100 seeds mass (g), and differences between sites: Sidi Ali was significantly different and lower than the other sites. Means±StDev.

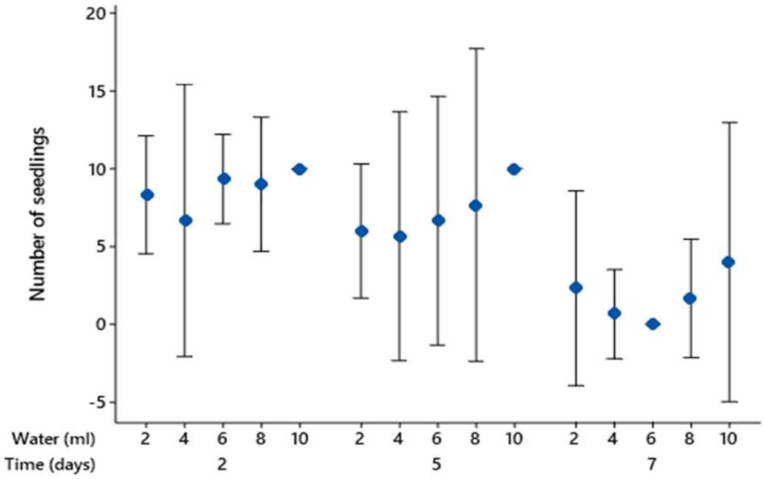

Figure 2. Number of seedlings versus water and time

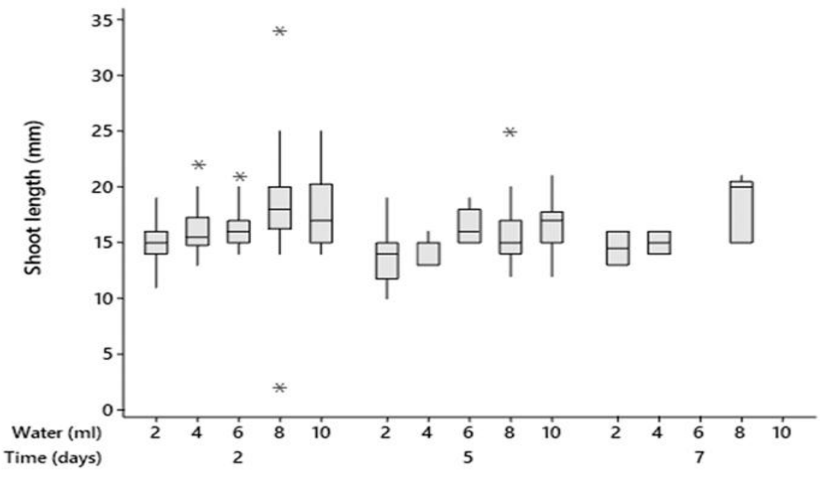

Figure 3. Shoot length versus water and time

Time does not have a significant effect on root length. Neither 2 days nor 5 days differ significantly from the baseline (7 days) (Fig. 4). Water strongly affects root length. Lower water levels (2 ml) significantly reduce root length, while higher water levels (8 ml) increase it substantially. Intermediate water levels (4 ml, 6 ml)

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do not differ significantly from the baseline (10 ml) (Fig. 4). The model explains only 12.93% of the variation in root length, indicating that other factors not included in the model may have a substantial influence. Optimal Conditions: Higher water levels (e.g., 8 ml or 10 ml) are recommended for longer root lengths. Lower water levels (e.g., 2 mL) should be avoided. No Significant Differences in root length among the three sites (p = 0.080). Thus, these differences are not statistically significant. However, Sidi Ali has the highest mean root length (4.5 mm), followed by Quarsha (4.4 mm) and Tika (3.7 mm) (Fig. 4).

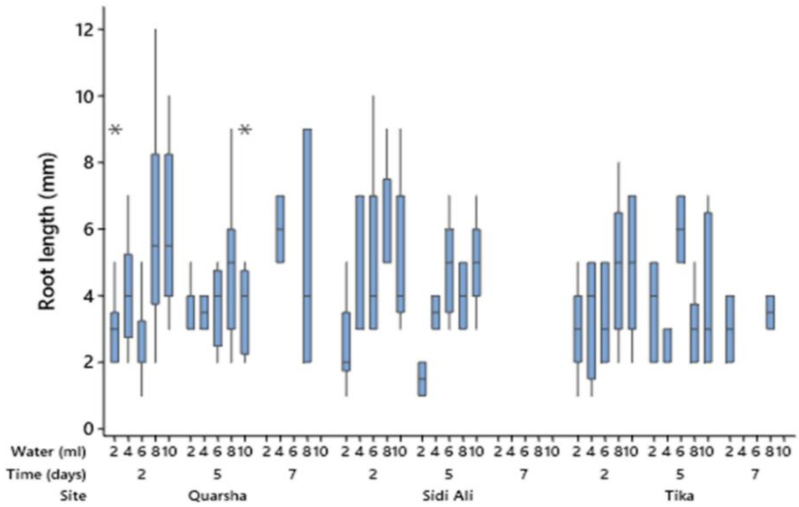

Figure 4. Root length versus site

Glasshouse experiment

There were no significant interactions between Time (days) and water (ml) in terms of effectiveness on shoot length, nor between Time and Water independently across all sites, except at site 2, Sidi Ali, where the effect of time was significantly different (Fig. 5). The GLM analysis reveals significant differences in shoot length across the three time points in site 2 (Sidi Ali), with the highest shoot length observed at 7 days. There were no significant interactions between Time (days) and water (ml) in terms of effectiveness on the root length, neither Time and Water independently over all sites, it seemed that quantity of water (10,15 and 20 ml) and period of watering time (2, 5 and 7 days) had no significant effect on the root length. Except, in general and across sites, there were significant differences in the amounts of water, as the quantities of 10 and 20 ml differed. In contrast, the amount of 15 ml was not significantly different from either (Fig. 6).

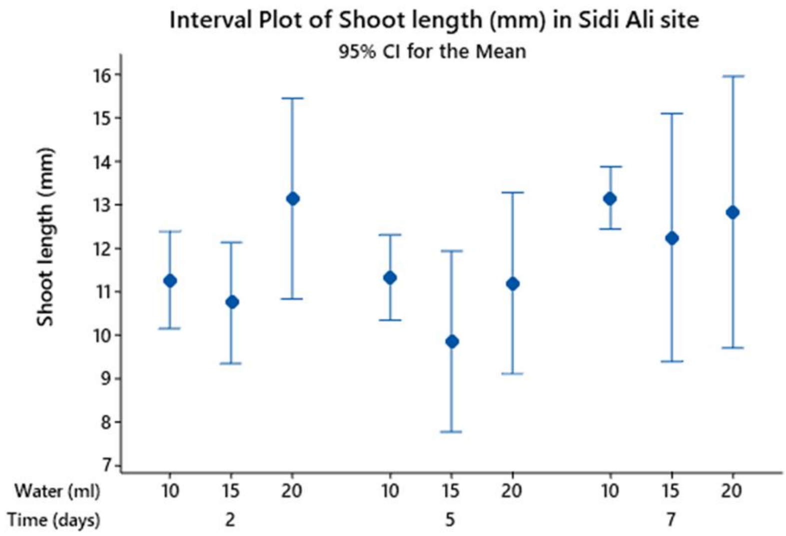

Figure 5. Shoot length in Sidi Ali site

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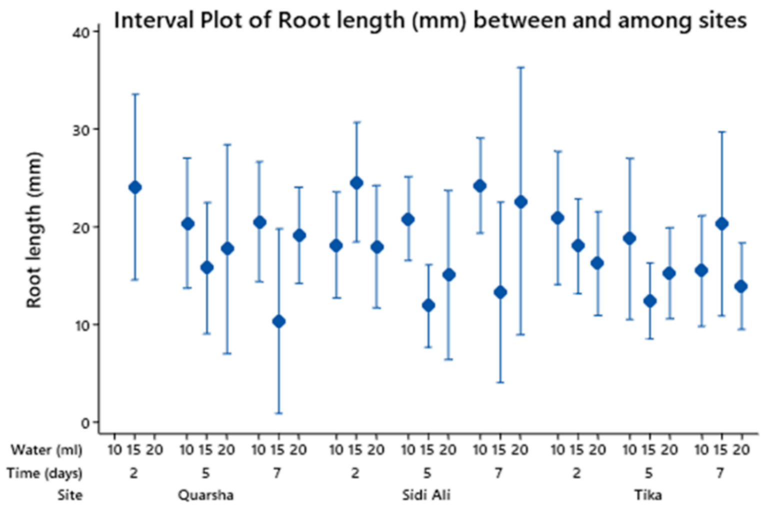

Figure 6. Effect of time and water on the root length of seedlings across sites

There were no significant interactions between Time (days) and water (ml) regarding their effectiveness on shoot dry mass. Similarly, neither Time nor Water independently affected the shoot dry mass over all sites. It appeared that the quantity of water (10, 10,15, and 20 ml) and the duration of watering time (2, 2,5, and 7 days) had no significant impact on shoot mass (Fig. 7).
In general, there were significant differences in the quantities of water across the sites. The quantities of 10 and 20 ml were different, while the amount of 15 ml did not differ from either.

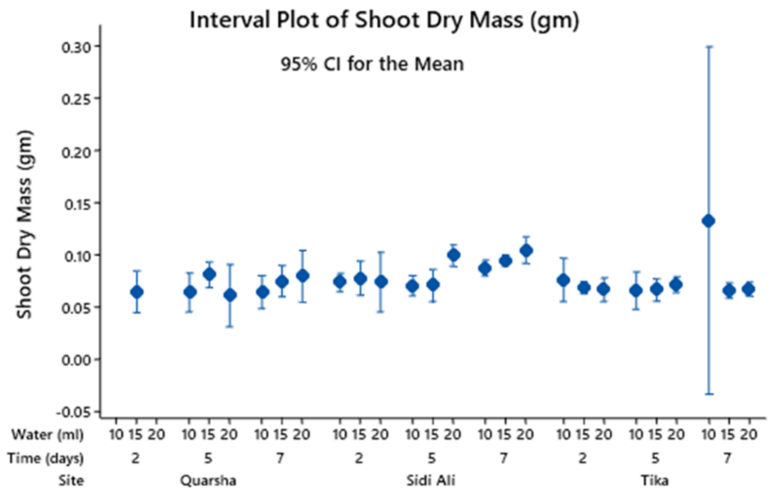

Figure 7. Effect of time and water on the shoot dry mass of seedlings across sites

There were no significant interactions between Time (days) and water (ml) regarding their effectiveness on root dry mass, nor between Time and Water independently across all sites. It appeared that the quantity of water (10, 10,15, and 20 ml) and the period of watering (2, 2,5, and 7 days) had no significant effect on root length and dry mass (Fig. 8).

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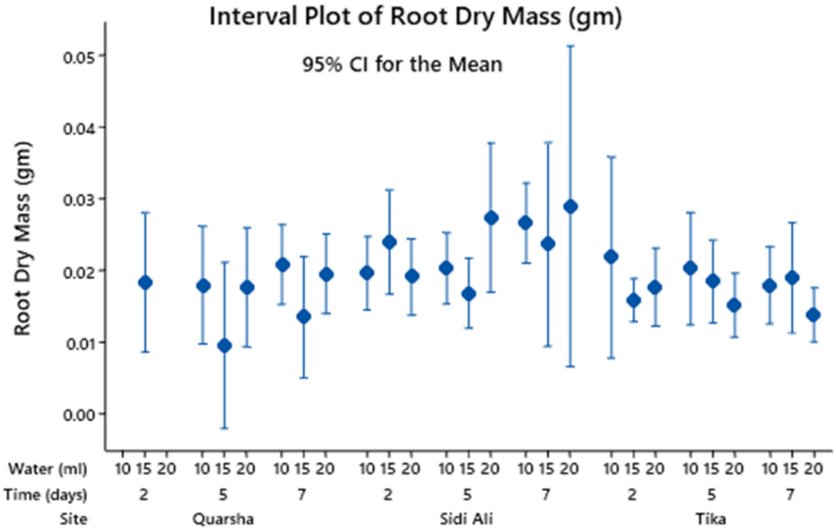

Figure 8. Effect of time and water on root dry mass of seedlings across sites

There were no significant interactions between Time (days) and water (ml) in terms of effectiveness on the Root/Shoot, neither Time and Water independently over all sites, it seemed that quantity io water (10,15 and 20 ml) and period of watering time (2,5 and 7 days) had no significant effect on the root length (Fig. 9).

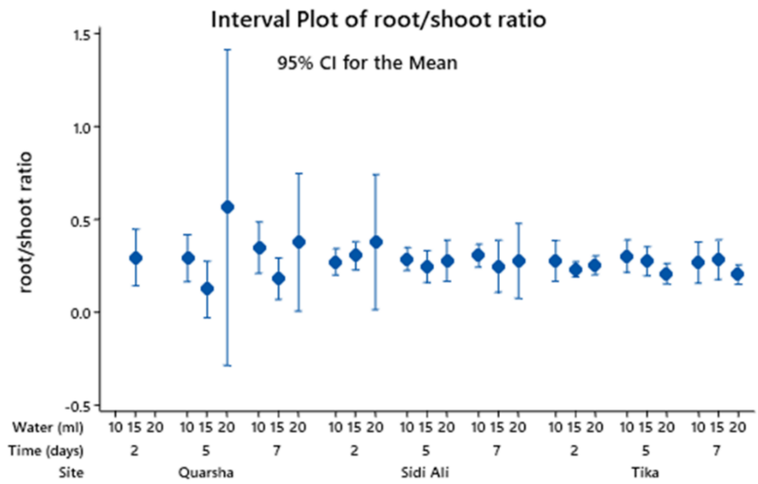

Figure 9. Effect of time and water on the Root/Shoot ratio of seedlings across sites

Discussion

This study offers important clues about the ability of this native tree to face various challenges in different environments. The carob tree, renowned for its resistance to drought and economic value, grows naturally in a Mediterranean climate. Nevertheless, it is threatened by climate change, human activity, and a decline in traditional agriculture [2]. Furthermore, this study demonstrated notable variability in germination success among carob seeds, influenced by water availability, seed mass, and site conditions. This finding supports earlier research emphasizing carob's adaptability to arid and semi-arid habitats, where water availability constrains plant growth [1,7].

The germination (Petri dish) experiment in this study demonstrated that mechanical scarification significantly improved germination rates, consistent with Zemouri et al. (2020), who identified seed coat dormancy as a significant barrier to carob germination [27]. Additionally, the study revealed that increasing the water supply (8–10 ml) enhanced both shoot and root growth, indicating that carob seedlings are particularly vulnerable to water stress during their initial growth phases. This is supported by Lo Gullo et al. (2003), who found that carob trees adopt drought-avoidance strategies, such as lowering stomatal conductance, to conserve water in dry conditions [28].

The study compared carob pods and seeds from Quarsha, Sidi Ali, and Tika from three Benghazi sites.

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Significant differences were observed across sites in pod length, mass, seed number, and seed mass. For instance, Tika had the highest mean pod length (16.2 cm) and seed number per pod (14.1), while Quarsha had the highest pod seed mass (2.2 g). These variations may be attributed to differences in soil quality, microclimate, and genetic diversity among carob populations [12,19].
The ANOVA test results confirmed that the site factor significantly influenced pod and seed characteristics, with Tika showing superior performance in most metrics. This suggests that Tika may provide more favourable conditions for carob cultivation, potentially due to its soil composition or microclimatic factors. However, further research is needed to identify the specific environmental variables driving these differences.
The glasshouse experiment in this study demonstrated that water availability significantly affects carob seedling growth. Shoot and root lengths were highest under moderate water regimes (8–10 ml), while lower water levels (2–4 ml) resulted in stunted growth. It was found that carob seedlings exhibit reduced leaf area and root development under water stress. The study also highlighted that root growth was more sensitive to water availability than shoot growth [29]. This may reflect carob's adaptation to drought-prone environments, where deep root systems are essential for accessing soil moisture [28].
Interestingly, the study found no significant differences in shoot dry mass across water regimes, suggesting that carob seedlings can maintain biomass production even under limited water availability. This resilience aligns with the species' reputation as a drought-tolerant crop [1].
The findings of this study have important implications for carob cultivation in Libya, particularly in the context of climate change and water scarcity. Carob's ability to thrive in poor soils and withstand drought makes it a valuable crop for sustainable agriculture in arid regions [7]. However, the decline in carob cultivation due to economic pressures and lack of management, as highlighted in the study, underscores the need for targeted interventions to promote its revival.
Regarding water management, the results suggested moderate irrigation (8–10 ml) is optimal for carob seedling establishment. This finding can inform irrigation strategies for carob orchards, ensuring efficient water use while maximising growth. Regarding the site, the variability of pod and seed characteristics across sites indicates that site-specific factors are crucial in carob productivity. Identifying and promoting sites with favourable conditions, such as Tika, could enhance carob cultivation in Libya. Most importantly, the success of mechanical scarification in improving germination rates highlights the importance of seed treatment techniques. Carob crop producers could adopt scarification methods to enhance seedling establishment and reduce germination failure. In terms of conservation and genetic diversity, the study underscores the need to conserve carob's genetic diversity, which is critical for its adaptation to changing environmental conditions. Initiatives such as seed banks and genetic resource management, as proposed by Di Guardo et al. (2019), could help preserve carob's resilience [30].
Finally, while this study provides valuable insights, it has some limitations. The experiments were conducted under controlled conditions, which may not fully replicate field conditions. Future research should include field trials to validate the findings and assess long-term growth and productivity. Additionally, the study focused on a limited number of sites and water regimes. Expanding the scope to include more sites and environmental variables would provide a more comprehensive understanding of carob's adaptability. Further research could also explore the role of mycorrhizal fungi in enhancing carob's drought tolerance [31], and investigate the potential of carob as a multipurpose crop for food, fodder, and environmental restoration.

Conclusion

This study highlights the potential of carob as a resilient and sustainable crop for arid regions such as Benghazi, Libya. By optimising water management, selecting suitable sites, and employing seed treatment techniques, carob crop producers can enhance carob cultivation and contribute to sustainable agriculture. However, addressing the challenges of climate change, economic pressures, and declining agricultural practices will require coordinated efforts from researchers, policymakers, and local communities. Carob's ecological and economic benefits make it a valuable asset for the Mediterranean region, and its conservation and promotion should be prioritised in agricultural development strategies.

Conflicts of Interest

The authors declare no conflicts of interest.

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