



The impact of altitude on seed germinability of libyan carob (*Ceratonia siliqua* L.) from Al-Jabal Al-Akhdar area

Rasha R Atiya¹, Eman M Abdalrasol², Sabah H Lamloom^{3*}

¹ Department of Biology, Education Faculty, Omar Al-Mukhtar University, Al Bayda', Libya

² Department of Biology, Education Faculty, Derna University, Darnah, Libya

³ Department of Botany, Science Faculty, Omar Al-Mukhtar University, Al Bayda', Libya

Abstract

Carob tree (*Ceratonia siliqua* L.) is an evergreen endemic species found naturally in Al-Jabal Al-Akhdar region which is located immediately south of the coastal belt in the northeastern region of Libya. The aim of the study was to investigate the effect of altitude on seed germination of *C. siliqua*. Seeds were collected from three different areas of Al-Jabal Al-Akhdar (Al-Aslab, Wadi Al-Kouf and Raas Al-Trab) with different altitudes (63, 360, and 669 m a.s.l., respectively). Mechanical scarification with soaking in distilled water treatment was used for the germination tests. Five replicates of 10 seeds from each region were placed inside a plant growth chamber and arranged in a completely randomized design. Germination parameters (germination percentage G%, mean germination time MGT and seedling length) were recorded. G% and seedling height were significantly decreased with the increased elevational gradients. In contrast, a slower rate of germination (i.e. high MGT) was observed at the higher altitude (Raas Al-Trab) region. The use of seeds from lower altitudes should be considered for seedling production of Libyan carob due to their higher germination potential.

Keywords: carob, *Ceratonia siliqua*, elevation and seed germination

Introduction

The carob tree is a slowly growing, woody evergreen, sclerophyllous, and widespread species occurring as a native plant in the Mediterranean Basin (Ramón-Laca & Mabberley, 2004) [19]. In Libya, carob tree is found naturally in Al-Jabal Al-Akhdar area which is situated instantly south of the coastal belt in the northeastern district of the country. Al-Jabal Al-Akhdar extends on the coast belt to about 300 km and rises to about 881 m above sea level. The average precipitation ranges between 250-600 mm, and the soils are terra-rossa or heavy clay (Johnson, 1973; Sharaf, 1971; El-Zwaam, 1995) [12, 22, 9]. The carob trees are dispersed in the area of Al-Jabal Al-Akhdar in association with many wild species such as olive (*Olea europaea*), mastic (*Pistacia lentiscus*) and juniper (*Junipurus phoenicea*), as cited in Ali *et al.*, (2019) [1].

Carob seeds are difficult to germinate and not willingly absorb water (Coit, 1951) [6]. Under natural conditions, only a reduced percentage of carob seeds are able to germinate. A number of factors (mechanical friction with soil particles, microbial action, passage through the digestive tract of mammals that feed on them, etc) can alter seed coat (Pérez-García, 2009) [18]. Previous studies (Lamloom and Abdalrasol, 2016a, 2016b) [13, 14] found that mechanical scarification and soaking in distilled water was the most effective method in increasing the germination percentages in *C. siliqua*.

Reproductive biology, mainly germination traits, is central to comprehending how species succeed with environmental because it could differ relying on altitude and other elements. Carob seedling restoration occurs even at high altitudes where the establishment and growth of plants happen in short periods. Some species can recruit through rapid germination during less severe climatic periods

variation (Baskin and Baskin, 2014; Bauk *et al.*, 2017) [2, 3]. A species may spread over the large geographical region and can grow in a broad range of situations with changing climates and topography. Consequently, an assessment of the germination capacity of seeds from different elevations is pertinent to define the reproductive effectiveness of a species (Vera, 1997) [23].

Thus, in this study, the seeds of Libyan carob from three different altitudes were exposed to mechanical scarification with soaking in distilled water treatment, which has been tried in previous studies to combat seed germination. Germinations parameters were then recorded and compared among three different regions.

Materials and Methods

Plant Material

Pods of carob trees were collected from three different regions of Al-Jabal Al-Akhdar (Al-Aslab, Wadi Al-Kuf and Raas Al-Trab) with different altitudes (63, 360, and 669 m a.s.l., respectively). Seeds were cleaned manually, placed in labeled paper bags and stored dry under laboratory conditions ($20 \pm 5^\circ\text{C}$) until the start of the experiment. Based on our observations from pre-sowing treatments (Lamloom and Abdalrasol, 2016a, 2016b) [13, 14], mechanical scarification & soaking in distilled water treatment was chosen for the germination tests. Seeds were scarified first and then soaked in distilled water for 24 h and disinfected under aseptic conditions to improve the germination percentage.

Five replicates of ten seeds per one region, with a total of 50 seeds per a region were germinated on top of two layers of filter paper (Whatman no. 1) in 15 cm diameters glass Petri dishes. Petri dishes were arranged in a completely randomize design inside a controlled - environment growth

chamber set at 90-96% relative humidity, 25±2°C under 16h-light and 8h-dark photoperiod with cool-white fluorescent lamps at 1500 lux. Germination was recorded daily and seeds were considered to have germinated when protrusion of the radicle was visible. Enough distilled water was added as needed to each Petri dish and filter papers were regularly moistened to ensure saturation throughout the germination tests. The experiments were terminated after 30 days.

Germination Parameters

Germination Percentage

The Germination Percentage was calculated according to the following equation (ISTA, 1999)

$$G \% = \text{seeds germinated} / \text{total seeds} \times 100.$$

Mean Germination Time

Mean germination time (MGT) was calculated by using the equation:

$$MGT = \sum(n \times d) / N,$$

where n = number of seeds germinated on each day, d = number of days from the beginning of the experiment, and N = total number of seeds germinated at the termination of

the experiment (Ellis and Roberts, 1981; Ranal and Santana, 2006) [8, 20].

Seedling Length

Seedlings length = root length + shoot length.

Statistical analysis

One-way analysis of variance (ANOVA) was performed to confirm the variability and validity of the data. Statistical analysis was performed using SPSS (Statistical Package for Social Sciences, version 22). The alpha level was set at 5%.

Results

G % and seedling lengths were significantly decreased with the increased elevational gradients. Figures 1 and 2 illustrated that lower elevation regions (Al-Aslab and Wadi Al-Kouf) had the highest germination percentages and seedling lengths. In contrast, high elevation region (Raas Al-Trab) had the lowest germination percentage and seedling lengths.

Furthermore, a slower rate of germination (i.e. high MGT) was observed at the higher altitude (Raas Al-Trab) region. Figure 3 showed that lower elevation regions (Al-Aslab and Wadi Al-Kouf) had the lowest mean germination time. In contrast, high elevation region (Raas Al-Trab) had the highest mean germination time (i.e. slower germination rate).

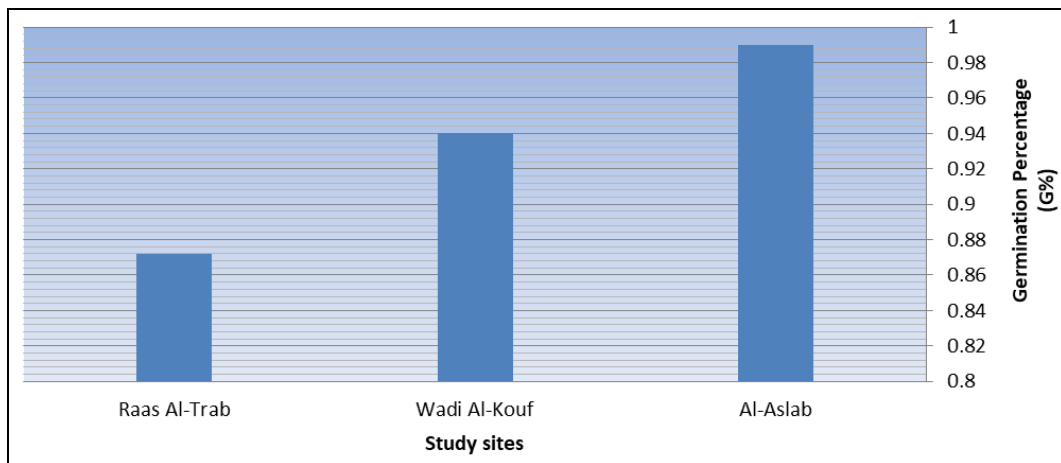


Fig 1: The effect of altitude on germination percentage (G%) of Wild *C. siliqua* from three studied sites.

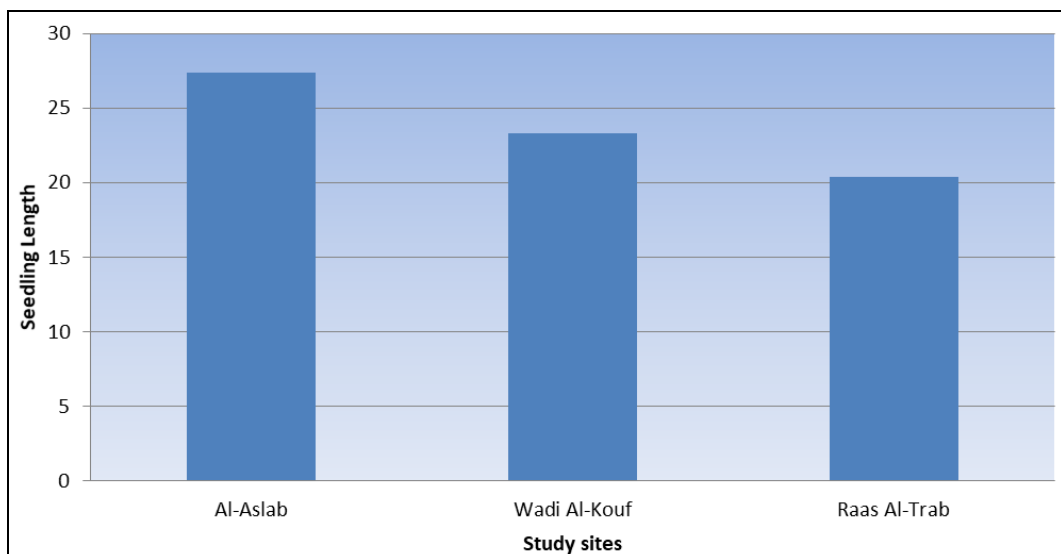


Figure 2: The effect of altitude on seedling length of Wild *C. siliqua* from three studied sites.

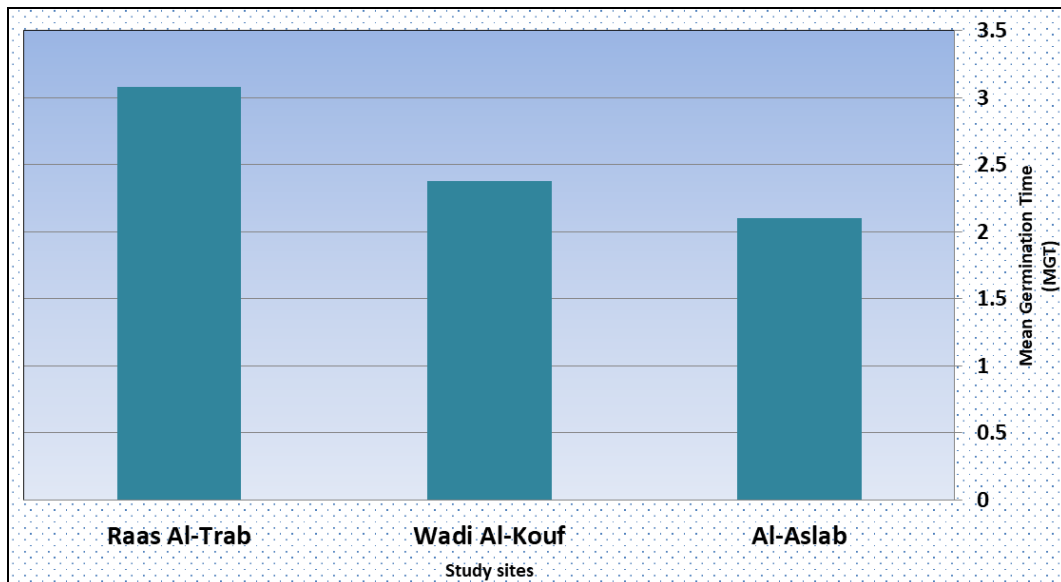


Fig 3: The effect of altitude on mean germination time (MGT) of Wild *C. siliqua* from three studied sites.

Based on the data of Table 1 and because the *p*-value of the independent variable, altitude, is statistically significant (*p* < 0.05), it is likely that altitude level does have a significant effect on G %, MGT and seedling length.

Table 1: Results of One-way ANOVA showing effect of altitude on germination percentage (G%), mean germination time (MGT) and seedling length of wild Libyan carob (*Ceratonia siliqua* L.) seeds collected from three regions with different altitudinal gradients.

Germination Parameters		Sum of Squares	df	Mean Square	F	P
G %	Between Groups	0.035	2	0.018	35.796	0.027
	Within Groups	0.006	12	0.000		
	Total	0.041	14			
Seedling Length	Between Groups	122.149	2	61.075	572.575	0.002
	Within Groups	1.280	12	0.107		
	Total	123.429	14			
MGT	Between Groups	2.548	2	1.274	112.412	< 0.001
	Within Groups	0.136	12	0.011		
	Total	2.684	14			

Discussion and Conclusion

Seed germination, as a significant feature of vegetation regeneration, is one of the most widely investigated topics in plant biology (Olff *et al.*, 1994; Vera, 1997; Baskin and Baskin, 2014) [17, 23, 2]. The capability of seed germination and seedling establishment can partially regulate the restoration of plant communities. The estimation of germination capacity is essential to describe the sexual reproductive efficiency of species and will offer an ecological background for succeeding searches of field dispersion. Geographically linked variation in seed germination is common amongst predominant plants (Lord, 1994) [16]. Populations in various environments may have different natural selection stress on germination performance. The elevation of the habitat is a probable element connected with seed germination differences among populations and species (Holm, 1994; Vera, 1997; Bu *et al.*, 2009) [10, 23, 5]. Seed germination is as well influenced by other growth circumstances for instance moisture and

temperature (Bewley, 1997; Liu *et al.*, 2017; Xu *et al.*, 2017) [4, 24, 15]. Temperature was revealed to directly or indirectly control germination rate and dormancy status.

Lately, mean germination time (MGT), the reciprocal of germination rate has been presented to be highly representative of emergence occurrence in seed lots. MGT utilized as a likely rapid and trustworthy assessment to rate the comparative emergence of seed lots (Roberts, 1988) [21]. Growth rates may decrease with increased altitude owing to diminished air and soil temperatures, shorter growing seasons, increased exposure to wind, and reduced supply of nutrients. Plants need carbon dioxide to grow, and lower air density and atmospheric pressure at high altitude produces lower carbon dioxide levels and a slower transpiration rate. Slow transpiration and restricted carbon dioxide retard the photosynthesis rate and growth.

At higher altitudes, lower temperature and a shorter growing season might decrease together photosynthetic rates and the energy accessible for seed development and establishment. Altitudinal gradients had an influence on seed germination, even though the effect was not constantly consistent among species (Holm, 1994; Vera, 1997) [10, 23].

The seed coat thickens and comprises more polyphenols with raising altitude. This manner seems to be accountable for the decreased permeability of the seed coat. High germination temperatures reduce the gas solubility in water and, at the same time, increase polyphenol oxidation in the seed coat. The embryos of seeds collected at low elevation thus receive a reduced O₂ flux. When seeds from high elevation exposed to high temperature, the O₂ flux is extremely diminished owing to the thicker seed coat and augmented levels of oxidizable polyphenols. There is a high association between germination and the mean of the average daily temperature for the 30 days prior harvest, high temperature preceding to harvest being correlated with high germination. Offspring of plants relocated from high to low elevation (and vice versa) designate the direct impact of the new setting on the seed coat obstruction of germination (Dorne, 1981) [7].

The goal of this study was to investigate the effect of altitude on breaking dormancy and stimulate seed germination of *C. siliqua*. Statistical analysis of results showed that there are significant differences between G%,

MGT and seedling length of each elevation, as seeds of higher elevation had slower and less germination in longer periods. So, changes in elevation are an effectual factor on seed germination features of this species and this factor has to be deliberated in seed provision and refurbishment with this species. The use of seeds from lower altitudes should be considered for seedling production of Libyan carob due to their higher germination potential.

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