Effects of Elevated Temperature on Carob (*Ceratonia siliqua* L.) Seed Germination from Al-Jabal Al-akhdar Area, Libya

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**Abstract:** This study was conducted on a wild carob genotype grown in Al-Jabal Al-Akhdar area (Balagrae, Al-Bayda, Libya). Seeds belonging to three individual trees chosen at random were collected and kept separately. To assess the effects of different temperature regimes on seed germination, mechanical scarification with soaking in distilled water treatment was used for the germination tests. Germination tests were performed in darkness in a temperature controlled incubators pre-set to six continuous temperature regimes (20, 25, 30, 35, 40 and 45°C). Different germination parameters were determined. The germinability of seeds under different temperatures indicated 25-30°C as optimum temperature for the germination of carob (*C. siliqua*) seeds. High temperatures (40 and 45°C) resulted in reduced measured germination parameters.

**Key words:** Carob • *Ceratonia siliqua* • Seed germination and elevated temperature

**INTRODUCTION**

Carob tree (*Ceratonia siliqua* L.), belongs to the Caesalpiniodeae sub family of the family Leguminoseae (Syn. Fabaceae). This species is normally dioecious and it is sclerophyllous evergreen tree that may grow up to 20 m in height under superlative environmental situations [1] but characteristically reaches heights of 8 to 15 m [2, 3].

Carob regarded as one of the most important indigenous species that comprise the plant cover of Al-Jabal Al-Akhdar area (Al-Wasita, Agfentta, WadiKouf, Al-Hania, Al-Hamama, Omar Al-Mukhtar, Messa, Alghariqa and North of Labraq). Carob trees spread naturally in all regions of Al-Jabal Al-Akhdar, it grows as pure carob populations or form mixed woodland together with *Juniperusphoenicea*, *Oleaeuropaea* L. var. oleaster, *Quercuscoccifera*, *Cupressus sempervirens* and *Pinushalepensis* [4].

Carob legumes (pods) are commonly used as animal feed or pulverized into flour and combined with other cereals for human expenditure. The legumes are rich in protein and sugar and a highly nutritious livestock feed, similar to barley and better than oats [5]. In addition, legumes are utilized in manufacturing health foods (as a chocolate substitute), carob syrup and medicines for instance laxatives and diuretics [6, 5]. As well, they can be used as an inexpensive carbohydrate resource for ethanol production. Carob seeds have galactomannan polysaccharides collectively identified as a carob, or locust-bean gum [1]; this compound is a precious stabilizing and thickening stabilizer used in the food processing pharmaceutical, textile, paper and petroleum industries. Carob gum is chiefly viscous and can be used rather than some gums [7].

Carob seeds are hard to germinate and not readily absorb water [6]. Under natural circumstances, only a reduced percentage of carob seeds are capable 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 change seed coat [8].

Temperature is one of the most significant environmental causes restraining germination [9, 10]. Germination speed usually increases until the temperature reaches 30-35°C [11]. Timing of germination can be monitored by the environmental temperature through the
temperature range over which germination may happen. This relationship is recognized as a mechanism that controls the dormancy condition of natural seed populations in innumerable species [9]. This kind of regulation by temperature permits the seeds to evade severe environmental circumstances for seedling establishment.

In Mediterranean surroundings, germination is preferential at moderately low temperatures, between 15°C and 20°C [12, 13 and 14]. Though, climatic conditions are altering, temperatures are escalating and are expected to continue increasing at a fast rate [15]. These upcoming climatic alterations are projected to have a major influence on biodiversity [16] and a number of modeling investigations have been started to forecast possible influences on plant dispersal [17, 18].

Global temperature is rising and that the chief cause is the buildup of carbon dioxide and other greenhouse gases in the atmosphere as a consequence of human activities [19]. Scientists as well affirm that climate change is a serious and growing threat and that the threat would become more rigorous over coming decades [20].

Climate change will not affect all taxa in the same way. Particular species and taxa will be more susceptible to its consequences than others because of discrepancy in exposure to climate change and biological variations between species [21]. Information of these differences can assist recognize vulnerable species that are priorities for conservation action and climate adjustment strategies [22].

Models of global climate change forecasts designate that an additional 1.1-6.4°C warming is anticipated for the Mediterranean basin throughout this century. Consequently, plants are expected to face increasing high temperature stress. This is drawing greater awareness in the direction of the investigation into plant reactions to these tense conditions [23, 24].

Yet, investigations on warming outcomes on Mediterranean species germination are lacking. Though declined germination with increasing temperatures possibly anticipated for plants growing in Mediterranean areas [12-14], there are no studies on germination response of carob seeds to temperature conditions from Al-Jabal Al-Akhdar area (Green Mountain), Libya. High temperature is one of the major abiotic stress factor constraining plant growth and productivity. The impact of climate change on temperature is of enormous importance in determining the future response of trees to the new environmental conditions. Information is required for the most important tree species of the Mediterranean to make long-term projections of their responses to these future climate warming. The research about the responses of C. siliqua to the typical stresses of the Mediterranean region assumes an increased importance in the context of the predicted future climatic changes.

Optimum temperature for carob seed germination was 25°C as was recorded by De Michele et al. [25] and 27.5°C by Mitrakos [26]. However, Pérez-García [8] indicated that there were no significant differences among different temperature treatments (10, 15, 20 and 25°C) tested. The seeds of the population investigated attained comparable final germination percentages at all temperature treatments. However, it notable that the different temperature treatments used by the researcher, all ranged from low to optimum temperatures.

Because Libya is one of the homeland regions of carob, it is important to conduct investigations into the seed germination of carob and the response of carob to the predicted rising temperature. Scant information is documented about the influences of the rising global temperature on seed germination in general. The current study is participation towards filling this gap of information and examines the ecophysiology of the germination performance in this sclerophyllous Mediterranean species.

This study was conducted on a wild carob genotype grown in Al-Jabal Al-Akhdar area (Balagrae, Al-Bayda, Libya). The major objective was (1) to examine the effects of the projected temperature rise on the germination characteristics of carob seeds and (2) to compare the germination responses of seeds belonging to different individual trees from the same site.

MATERIALS AND METHODS

Study Site: Balagrae (about 10 km south of Al-Baida, 32° 73’ – 32° 77’ N 21° 70’ – 21° 68’ E, 522.5 m height above sea level) was chosen as the study site because it is the most diversified woodland close to Omar Al-Mukhtar University campus.

The study was carried out at the graduate studies laboratory (Botany Department, Science Faculty) at Omar Al-Mukhtar University (Al-Baida, Libya) from August 2014 to June 2015.

Climatic Measurement: Study site undergo a typical Mediterranean climate, distinguished by dry, hot summer and mild, humid winter. Mean annual temperatures range between 11.9 and 21.4°C. Mean January temperature lies between 6.3 and 13.5°C, mean July temperature above 16.8°C, the highest value reached is 27.5 °C.

Climatological data issued from Al-Baida meteorological center was obtained from 1999-2015.
Seed Material:
- Ripe fruits (pods) containing mature seeds of *Ceratonia siliqua* were collected in August 2014 from a wild growing population in Balagrae from Al-Jabal Al-Akhdar area.
- Seeds belonging to three individual trees chosen at random from the study site were collected and kept separately. Seeds were cleaned manually, placed in labeled paper bags and stored dry under laboratory conditions (20 ± 5°C) until the start of the experiment.

Seed Viability Test: To test seed viability (is a measure of the percentage of seeds that are alive after storage), seeds were placed in a beaker then were soaked in water. The seeds that float up were discarded.

Control of Pathogens: Aseptic technique is an important way to reduce pathogenic contamination by fungi, molds and bacteria by killing and minimizing their presence. All work surfaces were cleaned and disinfected with 95% ethanol. The easiest way to prevent contamination is by surface sterilization of seeds to be used in the research. Seeds were soaked in 70% ethanol for 1 minute then were thoroughly rinsed 4 to 5 times in sterilized distilled water, to minimize microorganism development at the early stages of germination.

The main purpose of the experiment was to investigate the effects of different constant temperatures on carob seed germination from the studied area.

Based on our observations from pre-sowing treatments [27], 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. Germination tests were performed in darkness in a temperature controlled incubators pre-set to six continuous temperature regimes (20, 25, 30, 35, 40 and 45°C). Seed germination was under dark conditions because we were concerned in investigating only the effect of temperature on seed germination, avoiding the triggering effect of other factors such as light. In all trials, three replicates of 10 seeds for each temperature treatment from each tree were arranged in a completely randomized design and were tested for germination on top of two sheets of filter paper (Whatman no. 1) in 15 cm diameter glass Petri dishes. Seeds were believed to have germinated when its radicle extended at least 0.5 mm out of the seed [28]. Germinated seeds were counted every 24 hours until no germination occurred [29]. 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 run for a minimum of 3-4 weeks and were continued as long as the seeds were still germinating (maximum 30 days).

Germination Parameters:
- The Germination percentage (G%) was calculated using the following formula [30]:

\[
\text{Germination\%} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100
\]

- The mean germination time (MGT) was calculated by the expression of [31].

\[
\text{MGT} = \frac{\sum t_i}{\sum n_i}
\]

where \( t_i \): time from the start of the experiment to the \( i^{th} \) observation (day for the example; \( n_i \): number of seeds germinated in the \( i^{th} \) time (not the accumulated number, but the number correspondent to the \( i^{th} \) observation)

- The speed of germination was calculated using the following formula [32]

\[
\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2}{Y_2} + \frac{X_3}{Y_3} + \ldots + \frac{X_n}{Y_n}
\]

where; \( X_i, X_1 \) and \( X_n \) are number of seeds germinated on first, second and \( n^{th} \) day, respectively and \( Y_1, Y_2 \) and \( Y_n \) are number of days from sowing to first, second and \( n^{th} \) count respectively.

- Germination value as defined by Czbator[33] is the integral of final mean daily germination percentage (MDG) and Peak value (PV).

\[
\text{GV} = \text{MDG} \times \text{PV}
\]

Final MDG is the cumulative percentage of full seed germination at the end of the test divided by the number of days elapsed since sowing date. PV stands for the maximum cumulative germination percentage divided by the number of days to attain this percentage.

Measurements of Root and Shoot Lengths: Seedlings were taken from each petri dish and were divided into shoot and root fractions in order to measure root and shoot lengths. It was measured with a measuring scale and expressed in centimeters.

Measurement of Seedling Length:

\[
\text{Seedlings length} = \text{root length} + \text{shoot length}
\]
Measurements of Fresh and Dry Weight for Both Shoot and Root: Shoot and root of each seedling were wrapped with filter paper and weighed the fresh weight first then they were dried using the high temperature oven method (130°C for 1 h); ISTA [34] and were weighed to get the dry weight. These were measured using digital balance and expressed in grams.

Measurements of Fresh and Dry Weight of Seedlings: Fresh weight of the seedling = fresh root weight + fresh shoot weight, whereas the seedling dry weight = root dry weight + shoot dry weight.

Moisture Content: Moisture content of seeds was determined by the following formula given by ISTA [34]:

\[
\text{Moisture percentage} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100.
\]

Seedling vigour was calculated following the formula given by [35]:

\[
\text{Vigour Index} = \text{Germination}\% \times \text{Seedling length}
\]

Statistical Analysis: To test the effects of elevated temperatures on carob seed germination, data were analyzed using General linear model (GLM) procedure of SPSS (Statistical Package for Social Sciences, version 20), with tree, temperature and their interaction as factors and the different germination parameters as dependant variables. The main effect was determined using Duncan’s Multiple Range comparison (DMRT) [36] at 5% level of significance (P< 0.05). Percentage values were arcsine transformed prior to statistical analysis.

Table 1: The effects of different temperature regimes, individual trees and their interaction on germination percentage, mean germination time, speed of germination, mean daily germination, peak value and germination value of *Ceratonia siliqua* seeds.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Germination%</th>
<th>Mean Germination Time</th>
<th>Speed Germination</th>
<th>Mean Daily Germination</th>
<th>Peak Value</th>
<th>Germination Value</th>
</tr>
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<tbody>
<tr>
<td>Temperature regimes</td>
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<tr>
<td>20</td>
<td>84.7±0.9*</td>
<td>5.57±0.28*</td>
<td>1.87±0.10*</td>
<td>15.2±1.0*</td>
<td>15.7±0.9*</td>
<td>246.3±32.4*</td>
</tr>
<tr>
<td>25</td>
<td>95.9±0.1*</td>
<td>2.42±0.12*</td>
<td>4.29±0.19*</td>
<td>35.2±1.9*</td>
<td>39.5±1.8*</td>
<td>1409±142*</td>
</tr>
<tr>
<td>30</td>
<td>91.3±0.8*</td>
<td>3.60±0.25*</td>
<td>2.97±0.18*</td>
<td>23.6±2.2*</td>
<td>24.6±2.3*</td>
<td>622.0±111.0*</td>
</tr>
<tr>
<td>35</td>
<td>77.3±3.7*</td>
<td>5.20±0.45*</td>
<td>1.62±0.21*</td>
<td>11.7±1.9*</td>
<td>12.2±2.1*</td>
<td>175.1±53.1*</td>
</tr>
<tr>
<td>40</td>
<td>52.1±3.4*</td>
<td>7.80±1.12*</td>
<td>1.15±0.11*</td>
<td>9.9±1.1*</td>
<td>9.9±1.1*</td>
<td>108.1±20.3*</td>
</tr>
<tr>
<td>45</td>
<td>17.5±3.4*</td>
<td>8.95±1.09*</td>
<td>1.03±0.16*</td>
<td>6.2±1.0*</td>
<td>6.8±1.0*</td>
<td>50.1±16.5*</td>
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<tr>
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<td>&lt;.0001</td>
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<tr>
<td>T 1</td>
<td>71.4±7.5*</td>
<td>4.28±0.29*</td>
<td>2.33±0.22*</td>
<td>19.2±2.2*</td>
<td>20.8±2.5*</td>
<td>489±106*</td>
</tr>
<tr>
<td>T 2</td>
<td>68.3±7.4*</td>
<td>5.99±0.84*</td>
<td>2.16±0.34*</td>
<td>16.4±2.7*</td>
<td>17.2±3.1*</td>
<td>427±116*</td>
</tr>
<tr>
<td>T 3</td>
<td>69.8±5.5*</td>
<td>6.50±0.78*</td>
<td>1.98±0.32*</td>
<td>15.3±2.9*</td>
<td>15.9±3.0*</td>
<td>389±152*</td>
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<tr>
<td><em>P</em> value</td>
<td>0.2331</td>
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<td>0.0004</td>
<td>0.0049</td>
<td>0.0005</td>
<td>0.3683</td>
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<td>Tem*tree (<em>p</em> value)</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.0006</td>
<td>0.0003</td>
<td>0.0397</td>
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<tr>
<td><em>R</em>²</td>
<td>0.9768</td>
<td>0.8903</td>
<td>0.9749</td>
<td>0.9299</td>
<td>0.9466</td>
<td>0.8922</td>
</tr>
</tbody>
</table>

Values represent (mean ± standard error). Mean values within column followed by the same letters are not significantly ( P = 0.05, Two-way ANOVA).
Fig. 1: The effects of temperature regimes, individual trees and their interaction on germination percentage (a) and mean germination time (b) of *C. siliqua* seeds.

Table 1 and Figure (2a) illustrate that the speed of germination (SG) was highest in seeds incubated under 25°C (4.29) followed by 30°C (2.97). Yet, there was a significant difference between these two temperature regimes. Furthermore, the lowest speed of germination (1.03) was recorded at 45°C followed by (1.15) at 40°C. However, there were no significant differences between these two temperature treatments. In contrast, the speed of germination was increased by (1.87, 1.62) at 20 and 35°C respectively. Nevertheless, the speed of germination at 20°C was significantly different than at 35°C.

Table 1 and Figure (2b) demonstrate that the mean daily germination (MDG) was recorded maximum (35.2) at 25°C followed by (23.6) at 30°C. Nonetheless, there were significant differences between these two temperature regimes. The minimum mean daily germination (6.8) was attained at 45°C followed by (9.9) at 40°C and (11.7) at 35°C. The mean daily germination at 45°C was significantly different than 40 and 35°C, but there were no significant differences between 40 and 35°C. In contrast, the mean daily germination for seeds that were grown at 20°C was increased only by (15.2).

Fig. 2: The effects of temperature regimes, individual trees and their interaction on speed of germination (a) and mean daily germination (b) of *C. siliqua* seeds.
Fig. 3: The effects of temperature regimes, individual trees and their interaction on peak value (a) and germination value (b) of C. siliqua seeds.

Table 1 and Figure (3a) clarify that the highest peak value (39.5) was noticed in seeds grown at 25°C followed by (24.6) at 30°C. However, the peak value at 25°C was significantly different than at 30°C. The lowest peak value (6.8) was noted at 45°C followed by (9.9) at 40°C and (12.2) at 35°C. Nevertheless, the peak value at 45°C was not significantly different than 40°C and 35°C but the peak value at 45°C was significantly different than 35°C. In contrary, when seeds were incubated at 20°C peak value was increased only by (15.7).

Table 1 and Figure (3b) elucidate that the highest germination value (1409) was recorded at 25°C followed by (622.0) at 30°C. However, the germination value at 25°C was significantly different than 30°C. The lowest germination value (50.1) was attained at 45°C. When seeds were incubated at 20 and 35°C, the germination values were increased by (246.3, 175.1), respectively. Though there were no significant differences between them. In contrast, the germination value at 40°C was increased only by (108.1). Yet the germination value at 40°C was not significantly different than at 20, 35 and 45°C.

Moreover, table 1, Figures 1, 2 and 3 show that the individual trees had high significant influences on mean germination time (P<0.0001), speed of germination, (P = 0.0004), mean daily germination (P = 0.0049) and peak value (P = 0.0005). While, individual trees had no significant effect on both the germination percentage (P = 0.2331) and germination value (P =0.3683). The temperature regimes and the individual trees interaction significantly affected all studied traits (Table 1, Figures 1, 2 and 3).

Table 2 shows the effects of temperature regimes, individual trees and their interaction on the root length, shoot length, seedling length, root fresh weight, root dry weight, shoot fresh weight and shoot dry weight of seedlings. The analysis of variance indicated that temperature regimes had very high significant effect on all above-mentioned parameters (P<0.0001). Table 2 and Figure (4a) indicate that the longest values for root length (21.0 cm), shoot length (4.7 cm) and seedling length (25.7 cm) were found at 25°C compared to all other temperature treatments. The root length at 20°C was (18.7 cm) followed by (18.6 cm) at 30°C. Moreover, the root length at 25°C was significantly different than 20 and 30°C. The shortest root length (0.58 cm) was found at 40°C. When, seeds were grown at 35°C the root length was increased only by (12.3 cm).

Table 2 and Figure (4b) designate that the shoot length was recorded (4.2 cm) at 20°C followed by (4.1 cm) at 30°C. However, the shoot length at 25°C was significantly different than shoot length at 20 and 30°C. In contrast, when seeds were incubated at 35°C, the shoot length was increased only by (1.7 cm).

Table 2 and Figures (4c, 5, 6, 7, 8, 9 and 10) specify that the seedling length was documented (22.9 cm) at 20°C followed by (22.7 cm) at 30°C. Though, the seedling length at 25°C was significantly different than seedling length at 20 and 30°C. The lowest seedling length (14.0 cm) was observed at 35°C. Taken into consideration the fact that seeds were not able to develop into complete seedling at 40 and 45°C.
Table 2: The effects of different temperature regimes, individual trees and their interaction on root length, shoot length, seedling length, fresh root weight, dry root weight, fresh shoot weight and dry shoot weight of Ceratonia siliqua seeds.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Seedling length (cm)</th>
<th>Fresh root weight (g)</th>
<th>Dry root weight (g)</th>
<th>Fresh shoot weight (g)</th>
<th>Dry shoot weight (g)</th>
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<tr>
<td>20</td>
<td>18.7±0.6a</td>
<td>4.2±0.19b</td>
<td>22.9±0.7a</td>
<td>0.369±0.018b</td>
<td>0.034±0.001b</td>
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<td>25</td>
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<td>4.7±0.2a</td>
<td>25.7±0.2a</td>
<td>0.332±0.007b</td>
<td>0.032±0.006b</td>
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<td>30</td>
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<td>4.1±0.13b</td>
<td>22.7±0.5b</td>
<td>0.317±0.007b</td>
<td>0.031±0.001b</td>
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<td>0.049±0.002b</td>
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<tr>
<td>35</td>
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<td>1.7±0.07b</td>
<td>14.0±0.3c</td>
<td>0.270±0.023c</td>
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<td>40</td>
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<td>-</td>
<td>0.016±0.004d</td>
<td>0.004±0.001d</td>
<td>0.05±0.011d</td>
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<tr>
<td>T 1</td>
<td>14.3±1.9a</td>
<td>3.5±0.4a</td>
<td>21.1±1.3a</td>
<td>0.259±0.033a</td>
<td>0.026±0.003a</td>
<td>0.209±0.021a</td>
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<td>T 2</td>
<td>13.9±1.9a</td>
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<td>20.9±1.5a</td>
<td>0.275±0.037a</td>
<td>0.028±0.004a</td>
<td>0.183±0.026a</td>
<td>0.035±0.005a</td>
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<tr>
<td>T 3</td>
<td>14.6±2.1a</td>
<td>3.7±0.4a</td>
<td>21.9±1.4a</td>
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<td>0.025±0.003a</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(P value)</td>
<td>0.2524</td>
<td>0.7568</td>
<td>0.5521</td>
<td>0.1283</td>
<td>0.1300</td>
<td>0.3060</td>
<td>0.0007</td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.9882</td>
<td>0.9176</td>
<td>0.9368</td>
<td>0.9490</td>
<td>0.8883</td>
<td>0.9328</td>
<td>0.9143</td>
<td></td>
</tr>
</tbody>
</table>

Values represent (mean ± standard error). Mean values within column followed by the same letters are not significantly (α = 0.05, Two-way ANOVA).

Fig. 4: The effects of temperature regimes, individual trees and their interaction on (a) root length, (b) shoot length and (c) seedling length of C. siliqua seeds.
Table 2 and Figure (11a) display that the highest root fresh weight (0.369 g) was revealed at 20°C followed by (0.332 g) at 25°C and (0.317 g) at 30°C. Yet, root fresh weight at 20°C was significantly different than at 25 and 30°C. The lowest root fresh weight (0.016 g) was observed at 40°C. In contrast, when seeds were grown at 35°C, the root fresh weight was increased only by (0.270 g).

Table 2 and Figure (11b) indicate that the highest root dry weight (0.034 g) was found at 20°C followed by (0.032 g) at 25°C, (0.031 g) at 30°C and (0.029 g) at 35°C. Though, there were no significant differences between 20°C and these three temperature regimes. The lowest root dry weight was recorded at 40°C.

Table 2 and Figure (12a) show that the highest shoot fresh weight (0.26 g) was observed in seeds grown under 35°C followed by seeds grown at 30, 25 fresh weight at 35°C was not significantly different than at 25 and 30°C but there were no significant differences between the shoot fresh weight at 20 and 25°C. The lowest shoot fresh weight (0.05 g) was obtained at 40°C.

Table 2 and Figure (12b) demonstrate the highest shoot dry weight (0.049 g) was attained at 30°C followed by (0.046 g) at 35, (0.045 g) at 20 and (0.031 g) at 25°C. However, 30°C was not significantly different than 35 and 20°C but there was a significant difference between shoot dry weight at 25°C and 30°C. The lowest shoot dry weight (0.010 g) was noticed at 40°C.

Furthermore, Table 2, Figures (4, 11 and 12) show that the individual trees had no significant effect on all above studied parameters listed in Table 3 except both root fresh weight ($P = 0.0378$) and shoot fresh weight ($P = 0.0040$). The temperature regimes and the individual trees interaction had no significant effect on all studied parameters (Table 2, Figures 4, 11 and 12) except shoot dry weight ($P = 0.0007$).
Table 3 demonstrates that the effects of the temperature regimes, individual trees and their interaction on the seedling fresh weight, seedling dry weight, root moisture%, shoot moisture%, seedling moisture% and vigour index of the seedlings. The analysis of variance indicated that temperature regimes had very high significant effect on all above-mentioned parameters \((P<0.0001)\), while the level of significance for shoot moisture% was \((P = 0.0169)\).

Table 3 and Figure (13a) indicate that the highest seedling fresh weight \((0.590 \text{ g})\) was revealed at \(20^\circ\text{C}\) followed by \((0.571 \text{ g})\) at \(25^\circ\text{C}\), \((0.566 \text{ g})\) at \(30^\circ\text{C}\) and \((0.529 \text{ g})\) at \(35^\circ\text{C}\). However, seedling fresh weight at \(20^\circ\text{C}\) was not significantly different than \(25^\circ\text{C}\) and \(30^\circ\text{C}\). While, seedling fresh weight at \(20^\circ\text{C}\) was significantly different than \(35^\circ\text{C}\). The lowest seedling fresh weight \((0.061 \text{ g})\) was revealed at \(40^\circ\text{C}\).
Table 3: The effects of different temperature regimes, individual trees and their interaction on seedling fresh weight, seedling dry weight, root moisture, shoot moisture, seedling moisture and vigour of Ceratonia siliqua seeds.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Seedling fresh weight (g)</th>
<th>Seedling dry weight (g)</th>
<th>Root moisture%</th>
<th>Shoot moisture%</th>
<th>Seedling moisture%</th>
<th>Vigour index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature regimes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.590±0.014c</td>
<td>0.079±0.002c</td>
<td>90.6±0.6b</td>
<td>78.7±1.9a</td>
<td>86.5±0.4a</td>
<td>1945.7±63.5b</td>
</tr>
<tr>
<td>25</td>
<td>0.571±0.005bc</td>
<td>0.063±0.004b</td>
<td>89.8±0.2b</td>
<td>85.9±1.3b</td>
<td>89.0±0.7b</td>
<td>2447.0±20.9b</td>
</tr>
<tr>
<td>30</td>
<td>0.566±0.009bc</td>
<td>0.080±0.003b</td>
<td>90.1±0.4b</td>
<td>80.5±0.6b</td>
<td>85.9±0.4b</td>
<td>2073.5±45.9b</td>
</tr>
<tr>
<td>35</td>
<td>0.529±0.028b</td>
<td>0.075±0.005b</td>
<td>88.3±1.6b</td>
<td>82.2±1.2a</td>
<td>85.6±0.9b</td>
<td>1087.0±61.3b</td>
</tr>
<tr>
<td>40</td>
<td>0.061±0.012bc</td>
<td>0.014±0.003b</td>
<td>73.9±2.2b</td>
<td>78.7±3.1b</td>
<td>77.8±2.5b</td>
<td>-</td>
</tr>
<tr>
<td>45</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.0169</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

| Trees               |                           |                        |                |                 |                   |             |
| T 1                 | 0.468±0.052c              | 0.064±0.006c           | 86.5±2.1b      | 81.8±1.3b       | 85.2±1.0b         | 1901±126c   |
| T 2                 | 0.462±0.059c              | 0.063±0.008c           | 87.8±1.6b      | 79.5±1.7b       | 84.7±1.3b         | 1879±171c   |
| T 3                 | 0.460±0.054c              | 0.059±0.007c           | 85.3±2.1b      | 82.8±1.8b       | 85.1±1.7b         | 1884±168c   |
| P value             | 0.8738                    | 0.3882                 | 0.2135         | 0.2322          | 0.9440            | 0.9134      |
| R²                  | 0.9694                    | 0.9303                 | 0.8118         | 0.4738          | 0.5769            | 0.5769      |

Values represent (mean ± standard error). Mean values within column followed by the same letters are not significantly (α = 0.05, Two-way ANOVA).

Fig. 13: The effects of temperature regimes, individual trees and their interaction on (a) seedling fresh weight and (b) seedling dry weight of C. siliqua seeds.

Table 3 and figure (13b) demonstrate that the highest seedling dry weight (0.080 g) was found at 30°C followed by (0.079 g) at 20°C, (0.075 g) at 35°C and (0.063 g) at 25°C. Yet, the seedling dry weight at 30°C was not significantly different than 20 and 35°C. The seedling dry weight at 25°C was significantly different than the other three temperature regimes (20, 30 and 35°C). The lowest seedling dry weight (0.014 g) was obtained at 40°C.

Table 3 and figure (14a) indicate that the highest root moisture% (90.6%) was found at 20°C followed by (90.1%) at 30°C, (89.8%) at 25°C and (88.3%) at 35°C. There were no significant differences between these four temperature regimes. The lowest root moisture (73.9%) was observed at 40°C. Yet root moisture at 40°C was significantly different than all other temperature treatments.

Table 3 and figure (14b) designate that the highest shoot moisture% (85.9) was revealed at 25°C, whereas, the lowest shoot moisture (78.7%) was observed at both temperature regimes (20 and 40°C) followed by (80.5%) at 30°C. However, there were not significant differences between these three temperature regimes (20, 40 and 30°C). In addition, the shoot moisture at 35°C (82.2%) was not significantly different than all other temperature regimes.
Fig. 14: The effects of temperature regimes, individual trees and their interaction on (a) root moisture% (b) shoot moisture% and (c) seedling moisture of *C. siliqua* seeds.

Fig. 15: The effects of temperature, individual trees and their interaction on seed vigour index of *C. siliqua* seeds.

Table 3 and Figure (14c) show that the highest seedling moisture% (89.0) was obtained at 25°C followed by (86.5%) at 20°C, (85.9%) at 30°C and (85.6%) at 35°C. Nevertheless, there were no significant differences between these four temperature regimes. The lowest root moisture (73.9%) was observed at 40°C. However, root moisture% at 40°C was significantly different than all other temperature treatments.

Table 3 and Figure 15 indicate that the highest seed vigour index (2447.0) was found at 25°C followed by (2073.5) at 30°C and (1945.7) 20°C. Nevertheless, vigour index at 25°C was significantly different than at these two temperature treatments (20 and 30°C). There were no significant differences between vigour index at 20 and 30°C. The lowest vigour index was revealed at 35°C. It is remarkable that seeds did not develop into complete seedlings at 45°C for all above-mentioned parameters (seedling fresh weight, seedling dry weight, root moisture%, shoot moisture%, seedling moisture% and vigour index). However, for vigour index even at 40°C, seeds were not able to develop into complete seedlings.
Moreover, Table 3, Figures 13, 14 and 15 clarify that the individual trees had no significant effect on all above studied parameters. The temperature regimes and the individual trees interaction had no significant effect on all studied parameters (table 3, figures 13, 14 and 15) except seedling dry weight ($P = 0.0075$) and vigour index ($P = 0.0288$).

**DISCUSSION**

Seed germination is highly reliant on temperature because temperature is one of the essential prerequisites of this process. Though, the range of temperature in which seeds accomplish enhanced germination depends mainly on species and it is different for every species or cultivar [37]. The effect of temperature on seed germination is quite compound because it influences each phase of germination process in a different manner and is not independent of other factors [38]. According to Roberts [39], three different physiological processes during seed germination that are influenced by temperature were confirmed. Firstly, temperature accompanied by moisture content, controls the rate of degeneration in all seeds; secondly, temperature influences the speed of dormancy alteration in moist seeds; and, thirdly, temperature determines the germination rate in non-dormant seeds.

Carob is originated worldwide and so it is possible to have broad range of temperature adaptability for its seed germination. Consequently, it is remarkable in this work to investigate the response of carob seeds to the changing levels of temperature.

High temperature stress is identified as the increase in temperature further than a critical threshold for a period of time enough to produce permanent damage to plant growth and development [40]. The growth and development of plants includes a numerous number of biochemical reactions, all of which are susceptible to some extent to temperature [41]. Therefore, plants reactions to high temperature differ with the level of the temperature increase, its duration and the plant species. Seed germination is extremely dependent on temperature because temperature is one of the essential prerequisites of this process. Rising temperature between base and optimum temperatures improved the rate of germination and total germination percentage, but temperatures above optimum reduce the germination percentage [42]. In certain situations, plants grown under elevated temperature as well produce low quality seeds, which have poor germination and vigour. Hall [45] stated that the maximum threshold temperatures for germination and emergence are greater for warm-season than for cool-season annuals. The capability of plants to deal with extreme temperatures is a complicated process and is controlled by environmental factors and as well by the genetic capacity of the plant [41].

Our results indicate that germination percentage, mean germination time, speed of germination, mean daily germination, peak value and germination value were all recorded highest at 25°C and lowest at 45°C. Root length, shoot length and seedling length were all observed the longest at 25°C and shortest at 40°C. Shoot moisture%, seedling moisture% and seed vigour index were noticed maximum at 25°C and minimum at 40°C. However, the minimum seed vigour index was noted at 35°C because at 40°C seeds were not able to develop into seedlings. Root fresh weight, root dry weight, root moisture% and seedling fresh weight were all detected highest at 20°C and lowest at 40°C. Shoot dry weight and seedling dry weight were both recorded maximum at 30°C and minimum at 40°C. Shoot fresh weight was the only germination parameter that was observed highest at 35°C and lowest at 40°C. Thus, the germinability of carob seeds under different temperatures indicated that optimum temperature of 25-30°C favoured seed germination of *C. siliqua*. This is in agreement with earlier studies [25, 26], which stated that the optimum temperature for carob seed germination is 25 and 27.5°C, respectively.

Derived from our findings, high temperatures (40 and 45°C) did not allow adequate rate of germination due to cell death and embryo damage during the early stage of development. Growth of roots and shoots in carob seedlings was not inhibited at 30 and 35°C, a reduction of shoot growth, root growth and other measured parameters of germination was observed at 40°C. In contrast, seeds were not able to develop into complete seedlings at 45°C. In accordance to our results, Piramila *et al.* [44] observed that seed germination as well as vigour index was significantly reduced by high temperature. Similar to our results, [40, 45] found that high temperature decreased shoot dry weight in maize, millet and sugarcane.

Our study should be considered as a first report to clarify germination of carob seeds in relation to temperature increase across Al-Jabal Al-Akhdar area. More research is needed in order to explain the response of carob seeds germination to the anticipated rising temperature in a larger scale taking into consideration the eco-physiological characters.
Little information is known about the effects of the increasing global temperature on seed germination in general. Collecting data on the factors controlling carob seed germination can guide to a better awareness about *C. siliqua* distribution with the prediction of potential flora change as a result of the approaching climate change. Luna *et al.*[46] findings show that alterations in temperature can affect the germination of some species, especially the endemics. Carob is considered as one of the endemics in the Mediterranean area.

The results obtained in our research were based on a laboratory experiment that may not adequately represent field conditions. Unfortunately, the translation or expansion of the laboratory outcomes to field circumstances is not simple and can lead to invalid conclusions [47]. Physiological laboratory experiments are generally not intended to represent the natural environment where seed is actually adapted to germinate or not germinate. Under controlled laboratory experiment, by growing seeds in complete darkness we focused only on the effects of temperature excluding the triggering effects of any other factors such as light or humidity, etc. There was a control over the conditions that affect seed germination while in the field there is no control over the various factors that influence seed germination. The findings of our study may not generalize to those field natural everyday environments. However, field experiment has less control and so it may be very difficult to determine what factors were important in explaining the outcome [47].

In the current study, the germination responses were derived from seeds that were collected throughout one single year. Consequently, it is not identified whether the germination response attained here would have been different if seeds had been harvested during different years.

It has been clearly recognized that some tree species such as carob, which has broad geographic distribution, has developed definite geographic races. These differ physiologically relating to such characteristics as cold, drought and disease resistance, photoperiod requisites and length of growing season. Due to natural selection over a long period of time, these strains have evolved physiological processes adapted to specific environments and they often do not do well when transferred to other environments. These differences are genetically controlled and transmitted through the seed. Consequently, it is advantageous to use seed from a local source or from a similar climate and latitude if local seed does not exist. Furthermore, we studied only seeds from three individual trees from the same site. It is worthwhile investigating a larger number of trees from the same site and from different sites. Because even individual trees of a species growing side by side vary genetically and physiologically, as showed by variations in growth rate, wood density, turpentine yield and other characters. Understanding that these characters often are passed on to their offspring through the seed has given vast motivation to more cautious selection of seed trees as sources of seed for nursery planting. Yet the offspring from each superior tree are more or less heterozygous and reveal a broad range of variation in advantageous characters. If we could learn more about the physiological processes monitoring rate and quality of growth, it might become much easier to select desirable types both for parents and for planting stock [48].

**CONCLUSION**

Regarding the response of carob seeds to elevated temperature, it should be noted that our effort is an initial move to unravel the effect of incubation temperature on carob seed germination. The germinability of seeds under different temperatures indicated 25-30°C as optimum temperature for the germination of carob (*C. siliqua*) seeds. High temperatures (40 and 45°C) resulted in reduced measured germination parameters.

We have to be careful with the elucidation of our findings because of the low number of trees used in this study. Further investigations that would incorporate a large number of trees from more sites would be essential to resolve such relationship.

Field experiments of carob seed germination would be mandatory to confirm the findings derived from these laboratory experiments.

**REFERENCES**


42. Prasad, P.V.V., K.J. Boote and L.H. Allen Jr, 2006. Adverse high temperature effects on pollen viability seed-set yield and harvested index of grain-sorghum (Sorghum bicolor (L.) Moench) are more severe at elevated carbon dioxide due to higher tissue temperatures. Agricultural and Forest Meteorology, 139: 237-251.


