
RESEARCH ARTICLE

Heat Stress Effects on the Germination of *Brachychiton populneus* (Schott & Endl.) from Al-Jabal Al-akhdar Area, Libya

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Highlights

- Heat stress significantly affects seed germination and seedling growth of *Brachychiton populneus*.
 - Germination performance varies under three constant (20, 30, and 40 °C) and one alternating (25–35 °C) temperature regimes.
 - High temperatures reduce germination percentage and seedling vigor.
 - Optimum germination (93.33%) and the fastest mean germination time (3.75 days) occur at 25–35 °C with 24 hours of seed soaking.
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Heat Stress Effects on the Germination of *Brachychiton populneus* (Schott & Endl.) from Al-Jabal Al-akhdar Area, Libya

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Abstract

Optimum temperatures play a crucial role in improving seed germination and seedling establishment for both tropical and non-tropical trees, and they are essential for developing effective strategies for vegetation restoration. The present study investigated the effect of heat stress on the germination and seedling growth of *Brachychiton populneus* (Schott & Endl.). Germination tests were conducted over one month under three constant temperature conditions (20, 30, and 40 °C) and one alternating temperature regime (25–35 °C; 12 h/temperature), after soaking seeds in distilled water for 12 and 24 hours. Results indicated that the heat stress negatively affects germination percentage, mean germination time, mean daily germination, seedling length, seedling vigor index, fresh and dry seedling weight, and seedling moisture percentage. The optimum germination condition is observed at the alternating temperature (25–35 °C) after 24 hours of soaking, which yields the highest germination percentage (93.3%) and the shortest mean germination time (3.75 days). The results showed that the minimum germination temperatures for *B. populneus* seeds were 20°C, and the maximum (40°C). This study concludes that *B. populneus* seeds exhibit better germination performance under alternating temperatures, highlighting the species' adaptability to fluctuating thermal environments and providing valuable insights for its propagation and restoration in heat-prone regions.

Keywords: *Brachychiton populneus*; Heat stress; Seed germination; Temperature responses

INTRODUCTION

Seeds are considered the primary means of reproduction for restoring degraded forests and enhancing afforestation initiatives in different countries (Syamsuwida et al., 2020). However, most tree species encounter difficulties with seed germination, due to various abiotic factors, including high temperatures (heat stress), especially in light of the changing Mediterranean climate caused by different human activities (Kumar et al., 2024; Roy et al., 2024). Elevated temperatures in the initial stages of seed development reduce seed size, quantity, and fertility and impair seed germination and seedlings (Beveridge et al., 2024; Reed et al., 2022). In addition, temperatures affect the biochemical reactions and the activity of enzymes that regulate different metabolic processes (Xia et al., 2018). Therefore, optimal temperatures for seed germination are determined by the environmental conditions to which the seeds have adapted in their native surroundings (Hsu et al., 2024). For example, Liu et al. (2024) found that the optimal temperature for the germination of *Quercus marlipoensis* tree seeds is 25 °C, according to the match of their habitat's climate.

Brachychiton is a genus comprising approximately 36 species within the Sterculiaceae family. It is noted for its ornamental trees that produce appealing bell-shaped flowers, commonly referred to as kurrajong or bottle trees (Guymer, 1988). In Australia, these species have been utilized as a food source and in traditional remedies for conditions such as diabetes, dermatitis, and gastrointestinal issues, as well as for their antiulcer and anti-allergic properties (Thabet et al., 2018a; Thabet et al., 2018b). Among these is *Brachychiton populneus* (Schott & Endl.) R. Br. has been introduced to Al-Jabal Al-Akhdar region of Libya (Salih et al., 2019). These reach heights of up to 20 meters and are native to tropical and subtropical regions of Australia (Buist et al., 2000). These trees possess robust trunks and roots that enable them to store water during dry periods (Franklin, 2016). Recent studies have indicated that *B. populneus* exhibits efficacy against plant-pathogenic fungi (Abdulrazziq et al., 2023). This activity may be attributed to the presence of polyphenolics, flavonoids, alkaloids, glycosides, sterols, and coumarins (Batool et al., 2019). Despite being *B. populneus* found in the Al-Jabal Al-Akhdar region, there is no information about

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their germination under different temperature conditions. Notably, *B. populneus* seeds exhibit physical dormancy at maturity, making it impossible to germinate without treatments (Salih et al., 2019). Correspondingly, this study represents the first attempt to evaluate the impacts of heat stress on the germination of *Brachychiton populneus* (Schott & Endl.) R. Br. under different temperatures.

MATERIALS AND METHODS

Brachychiton populneus seeds were randomly harvested from the mature pods of growing trees in the inner garden of Al-Thawra Hospital during 2024, in Al-Bayda city. This city is located northeast of Libya, 522.5 meters above sea level, between longitudes 21° 49' and 21° 41' E and between latitudes 32° 44' and 32° 47' N. The city is about 1142.9 hectares. In general, the climate of Al-Bayda city is a Mediterranean climate. The annual highest and lowest temperatures are 9°C and 23.6 °C, respectively. It was noticed that the highest rainfall from 1985- 2019 at 563 mm yr⁻¹ (Abd El Galil et al., 2022).

Plant materials and experimental design.

The vitality of the seeds was evaluated by submerging them in distilled water to remove any non-viable seeds. seeds underwent surface disinfection through immersion in a 5% sodium hypochlorite solution for 5 minutes, and then washed in distilled water (Luzia Delgado et al., 2015). Treatments consisted of seeds not soaked (control), and seeds soaked in distilled water for 12 and 24 hours, after soaking in sulfuric acid 96% for 30 minutes (Salih et al., 2019). Seeds were prepared for germination at three constant temperatures (20, 30, and 40 °C) and alternating temperatures (25-35 °C, 12 h/ temperature). The germination was performed under dark conditions in pre-set incubators. The experiment was conducted in the Biology Department laboratory at the Faculty of Education, Omar Al-Mukhtar University. The experiment was carried out with a completely randomized design (CRD) with three replicates as follows:

1. Untreated (control) seeds at constant temperatures (20, 30, and 40 °C) and alternating temperatures (25–35 °C).
2. Seeds soaked in distilled water for 12 h + constant temperatures (20, 30, and 40 °C) — (W12h + °C).
3. Seeds soaked in distilled water for 24 h + constant temperatures (20, 30, and 40 °C) — (W24h + °C).
4. Seeds soaked in distilled water for 12 h + alternating temperature (25–35 °C) — (W12h + 25–35 °C).

Each Petri plate (diameter 9 cm) with two Whatman No. 1 filter papers contained 25 seeds/plate. Filter papers were moistened with 10 mL of distilled water. These plates were incubated at 20, 30, 40, and 25-35 °C. The seeds were considered germinated at the exit of the radicle outside the seed cover, and germinations were counted daily for one month.

Germination parameters

The following germination and seedling growth parameters were evaluated: germination percentage (GP%), mean germination time (MGT), mean daily germination (MDG),

seedling vigor index (SVI), seedling moisture percentage, seedling length, and fresh and dry seedling weight. These parameters were calculated using the following formulas:

Germination percentage (GP%) = (Number of germinated seeds / Total number of seeds sown) × 100 (Salih & Abdulrazziq, 2024).

- Mean germination time (MGT) = Σ (Number of seeds germinated each day × Days) / Total number of germinated seeds (Salih & Abdulrazziq, 2024).
- Mean daily germination (MDG) = Final germination percentage / MGT (Nasr et al., 2013).
- Seedling vigor index (SVI) = Germination percentage × Mean seedling length (Abdul-Baki & Anderson, 1970).
- Seedling moisture percentage = [(Fresh weight – Dry weight) / Fresh weight] × 100 (Abdul-Baki & Anderson, 1973).
- Seedling length was measured using a millimeter ruler and expressed in centimeters. Fresh and dry weights of seedlings were determined using a digital balance and expressed in grams. Each seedling was wrapped in filter paper and weighed to determine its fresh weight. Subsequently, seedlings were oven-dried at 130 °C for one hour and reweighed to obtain dry weight (ISTA, 2003).

Statistical analysis

The statistical analysis was performed using (Minitab 17 program, and ANOVA variance analysis tables were compared to averages using Tukey's test at P<0.05.

RESULTS

The results presented in Table 1 indicate that different heat stress levels produced significant ($p \leq 0.05$) effects on the germination percentage, mean germination time, mean daily germination, and seedling length of *B. populneus* after 30 days of incubation. The highest germination percentage (93.3%) and the shortest mean germination time (3.75 days) were recorded at the alternating temperature treatment (W24h + 25–35 °C). No statistically significant differences were observed among treatments W12h + 30 °C, W24h + 30 °C, and W12h + 25–35 °C, which showed similarly high germination percentages (88.0%, 90.0%, and 88.0%, respectively) and mean germination times (3.78, 3.21, and 3.96 days, respectively).

In contrast, germination percentage declined to 73.3% and 76.0%, with slightly delayed mean germination times of 4.53 and 4.08 days at W12h + 20 °C and W24h + 20 °C, respectively. The lowest germination percentages (52.0% and 58.6%) and the longest mean germination times (6.58 and 6.87 days) were observed at W12h + 40 °C and W24h + 40 °C, indicating severe inhibition under high-temperature stress.

Mean daily germination followed a similar pattern, reaching a maximum of 28.03 at W24h + 30 °C, followed by 24.88 at W24h + 25–35 °C, 23.28 at W12h + 30 °C, and 22.22 at W12h + 25–35 °C. Lower values were observed at cooler temperatures (18.62 at W24h + 20 °C and 16.18

at W12h + 20 °C), with the lowest rates recorded at high temperatures (7.90 and 8.53 at W12h + 40 °C and W24h + 40 °C, respectively).

Seedling length also varied significantly among treatments. The longest seedlings (19.2 cm) were obtained from seeds germinated at W24h + 25–35 °C, followed by 17.4 cm at W12h + 25–35 °C, and 15.0 and 15.3 cm at W12h + 30 °C and W24h + 30 °C, respectively. The shortest seedlings (13.2 and 13.9 cm) were recorded at W12h + 20 °C and W24h + 20 °C. Notably, seeds failed to produce seedlings at the highest temperature treatments (W12h + 40 °C and W24h + 40 °C).

The data presented in Table 2 shows that heat stress levels affect seedling vigor index, fresh seedling weight, dry seedling weight, and seedling moisture percentage. There were highly significant differences between heat stress levels in all of these parameters for *B. populneus* seeds after 30 days of germination. The results showed that the maximum seedling vigor index (1791.9) was recorded at W24h+25-35°C, followed by (1531.2) at W12h+25-35°C (1377.0), W24h+30 °C, and (1320.0) at W12h+30 °C. The lowest seedling vigor index was recorded at (967.9 and 1056.4) at two temperatures, W12h+20°C and W24h+20°C, respectively. Nevertheless, seeds exposed to W12h+40 °C and W24h+40°C did not exhibit a seedling

vigor index, as they could not develop into complete seedlings. Fresh seedling weight from *B. populneus* was significantly affected by heat stress levels (Table 2). Where the seeds grown below W24 h +25-35°C had the greatest fresh seedling weight of (0.785g), followed by (0.670g) at W12h+25-35°C, (0.576g) at W24h+30°C, and (0.526g) at W12h+30°C. The lowest fresh seedling weight (0.335, 0.373g) was observed at W12h+20 °C and W24h+20 °C, respectively. The statistical analysis indicated no significant difference in the dry seedling weight values (0.082 and 0.079g) between seeds cultured at W12h+25-35°C and W24h+25-35°C. Likewise, between seeds cultured at the two temperatures, W12h+30°C and W24h+30°C, the dry weights were recorded as (0.068g) and (0.072g), respectively. Conversely, the minimum dry seedling weight (0.054, 0.050g) was recorded in seeds exposed to W12h+20°C h and W24h+20°C, respectively. However, there was no significant difference between these two temperatures. Also, table (2) designates that the highest seedling moisture percentage of (89.5%) was revealed at W24h+25-35 °C, followed by (88.2%) at W12h+25-35°C. There were no statistical differences between both W12h+30°C and W24h+30°C, with (87.0%) and (87.5%), respectively. The lowest seedling moisture percentage (83.8, 86.5%) was observed at both W12h+ 20°C and W24h+ 20°C, respectively.

Table 1. Effects of heat stress on germination percentage, mean germination time, mean daily germination, and seedling length of *Brachychiton populneus* seeds.

Temperature (°C)	% Mean Germination	Mean germination time (days)	Mean daily germination	Seedling length (cm)
Control	00.0 g	0.0 c	00.0 g	00.0 e
W12h+°C	20	73.33± 2.3 ^d	4.53±0.38 ^b	16.18±0.20 ^e
	30	88.00±4.0 ^b	3.78±0.21 ^b	23.28±0.28 ^{bc}
	40	52.00±0.0 ^f	6.58±0.58 ^a	7.90±0.10 ^f
	25-35	88.00±0.0 ^b	3.96±0.26 ^b	22.22±0.22 ^c
W24h+°C	20	76.00± 4.0 ^c	4.08±0.21 ^b	18.62±0.03 ^d
	30	90.00±0.7 ^b	3.21±0.15 ^b	28.03±0.30 ^a
	40	58.66±2.3 ^e	6.87±0.74 ^a	8.53±0.05 ^f
	25-35	93.33±2.3 ^a	3.75±0.19 ^b	24.88±0.08 ^b

*Values represent mean ± standard error. Means followed by the same letter within a column are not significantly different according to Tukey's test at the 5% probability level.

Table 2: Effects of heat stress on seedling vigor index, fresh seedling weight, dry seedling weight, and seedling moisture percentage of *Brachychiton populneus* seeds.

Temperature (°C)	Seedling Vigor Index (SVI)	Fresh Seedling Weight (g)	Dry Seedling Weight (g)	Seedling Moisture %
Control	0 g	0.0 g	0.0 d	00.0 e
W12 h+ °C	20	967.9 ±8.0 ^f	0.335±0.01 ^f	0.054±0.04 ^c
	30	1320.0±41.3 ^d	0.526±0.006 ^{cd}	0.068±0.002 ^b
	40	0±0.0 ^g	0.0±0.0 ^g	0.0±0.0 ^d
	25-35	1531.2 ±13.0 ^b	0.670±0.08 ^b	0.079±0.020 ^a
W24 h+ °C	20	1056.4±16.4 ^e	0.373±0.02 ^e	0.050±0.030 ^c
	30	1377.0±15.2 ^c	0.576±0.01 ^c	0.072±0.010 ^b
	40	0±0.0 ^g	0.0±0.0 ^g	0.0±0.0 ^d
	25-35	1791.9±12.0 ^a	0.785±0.03 ^a	0.082±0.050 ^a

DISCUSSION

Seed germination is a critical phase in the plant life cycle, and its success depends largely on the seed's ability to sprout under favorable environmental conditions (Puglia, 2024). Heat stress significantly alters physiological, biochemical, and molecular processes, often inhibiting or delaying seed germination (Ren et al., 2018). Moreover, the projected rise in global temperatures during the twenty-first century poses additional challenges for the growth and establishment of tropical tree species (Reis et al., 2021a). Understanding the optimal temperature requirements for seed germination and seedling establishment is therefore vital, particularly for species introduced or cultivated outside their native habitats (Ordoñez-Salanueva et al., 2021).

In this study, the germination behavior of *Brachychiton populneus* seeds introduced to the Al-Jabal Al-Akhdar region of Libya was evaluated under three constant temperature conditions (20, 30, and 40 °C) and one alternating temperature regime (25–35 °C, 12 h per temperature), following seed soaking in distilled water for 12 and 24 hours. The findings indicate that the optimum germination occurs at the alternating temperature treatment (W24h + 25–35 °C), producing the highest germination percentage and shortest mean germination time. This was followed by W24h + 30 °C, W12h + 30 °C, and W12h + 25–35 °C, which showed statistically similar performance.

These results are consistent with the findings of Oliveira et al. (2020), who reported that alternating temperatures between 25–35 °C yielded the highest germination vigor in *Callisthene* major var. seeds. Similarly, several studies have confirmed that the optimal germination temperature for many tropical and subtropical species is approximately 30 °C (Atalde et al., 2017; Benedito et al., 2019; de Freitas et al., 2024). This optimum temperature promotes rapid and uniform germination by balancing the levels of abscisic acid and gibberellins, enhancing water uptake, cellular respiration, and metabolic reactivation, as well as stimulating hydrolytic enzyme activities such as amylases, lipases, and proteases (Tsfay et al., 2016; Zuo & Xu, 2020).

Conversely, at 20 °C, germination percentage was moderate and germination was delayed, resulting in lower overall germination performance compared with the optimal temperatures. Increasing the temperature to 40 °C induced heat stress, leading to the lowest germination percentage and mean daily germination rate, along with extended germination time and visible damage to seed morphology. No normal seedlings were produced at this temperature, indicating its inhibitory effect on germination. Similar inhibitory responses at supra-optimal temperatures have been reported for *Buchenavia tomentosa* and *Ormosia coarctata* seeds, where exposure to 40 °C impeded both germination and seedling establishment (Correa et al., 2020; Reis et al., 2022).

This adverse effect of high temperature may be attributed to the accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) within cellular organelles such as chloroplasts, mitochondria, and the endoplasmic

reticulum, leading to oxidative stress (Thiruvengadam et al., 2024). Consequently, heat stress reduces antioxidant enzyme activity and lipase function, thereby impairing germination and early seedling development (Reis et al., 2021b).

CONCLUSIONS

Introducing tropical forest species for ornamental purposes or as a source of timber can play an important role in enhancing local biodiversity and supporting ecological restoration. The present study demonstrated that *Brachychiton populneus* seeds exhibit the highest germination success under an alternating temperature regime (W24h + 25–35 °C), which produced the greatest germination percentage and the shortest mean germination time. Seeds exposed to W24h + 30 °C, W12h + 30 °C, and W12h + 25–35 °C also germinated well, showing statistically similar performance, while lower or supra-optimal temperatures (20 °C and 40 °C) significantly reduced germination and inhibited normal seedling development. These results highlight the importance of providing suitable thermal conditions to maximize germination and seedling vigor in introduced species. Understanding the optimal temperature requirements of *B. populneus* can guide propagation efforts, improve seedling establishment, and facilitate successful cultivation of this species in non-native regions, particularly under changing climatic conditions.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHOR CONTRIBUTION

Conceptualization, S.M.; Data collection, Statistical analysis: S.M., A.A.; Data interpretation: S.M., A.A.; Writing the first draft: S.M., A.A.; Manuscript editing and reviewing: S.M., A.A.; Both authors read and approved the final manuscript.

DATA AVAILABILITY

Data are available upon request from the corresponding author.

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