

Effect of temperature on seed germination of five hemp (*Cannabis sativa* L.) cultivars from Rif Mountains (northern Morocco)

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Abstract: Seed germination is a critical early factor influencing crop yield and quality. Hemp (*Cannabis sativa*) seed germination is significantly affected by changes in temperature and light conditions. This study investigates the effect of temperature on the germination of five cannabis cultivars from the Fifi region (northwestern Morocco), specifically 'Beldiya', 'Mexicana', 'Khardala', 'Avocat', and 'Critical Plus'. We examined how temperature impacts several agro-biological parameters, including the final germination percentage (FGP), total germination time (TGT), mean germination time (MGT), mean daily germination (MDG), and latent life span (LLS). Our findings show that high temperatures (30°C) adversely affect all germination parameters across cultivars. The optimal FGP was observed within the 20°C to 25°C range for all cultivars. As temperature increases, germination accelerates, reducing TGT by 4 to 6 days depending on the cultivar. Rising temperatures also decrease MGT, grouping the cultivars into two response categories. In response to temperature shifts from 10°C to 25°C, 'Beldiya', 'Mexicana', and 'Critical Plus' exhibited significant reductions in MGT by 1.90, 1.83, and 1.80 days, respectively. 'Khardala' and 'Avocat' responded moderately, with decreases of 1.41 and 1.50 days. Higher temperatures also boosted MDG across all cultivars. At 10°C, MDG values increased by a factor of 2.4 for 'Beldiya', 2.6 for 'Khardala' and 'Avocat', 3.4 for 'Critical Plus', and 4 for 'Mexicana'. Furthermore, increasing temperatures shortened the LLS, advancing germination. Between 10°C and 25°C, 'Mexicana' and 'Critical Plus' advanced germination by 2.8 to 3.5 days, while 'Avocat', 'Beldiya', and 'Khardala' advanced by 1.7 to 1.8 days. Overall, the local cultivars demonstrated better adaptation to local environmental conditions compared to newly introduced varieties.

Keywords: Hemp; germination tests; *Cannabis*; Fifi; Morocco; Hemp cultivars.

Introduction

Man has utilized the fibers, resins and seeds of hemp (*Cannabis sativa* L.) since ancient times (Courtwright, 2002). Its applications date back to the dawn of civilization, serving not only utilitarian and agricultural purposes but also providing medicinal and psychoactive benefits (Chouvy, 1997; Zuk-Golaszewska and Golaszewska, 2018; El Ghacham et al., 2023). Evidence of hemp use has been found in China dating back over a thousand years before our era, marking the beginning of its spread to Africa and Europe in the 16th century and later to the Americas in the 17th century (Grotenhermen, 2009). The Arabs played a significant role in introducing hemp to the Western world, primarily for fabric and rope production (Marino, 2015). The psychoactive properties of the plant were recognized long before the Christian era; by the 15th century BCE, the Chinese were using it to alleviate rheumatic pain, treat gout, and as both an antiemetic and anesthetic (Decorte et al., 2011).

In Morocco, cannabis cultivation is primarily concentrated in the Rif Mountains, located in the northern part of the country. Initially,

it was grown for the production of both kif (a chopped cannabis herb smoked in pipes) and hashish (a resin-based drug) (Chouvy and Afsahi, 2014). The cultivation of hemp has a long history in the region, dating back to between the 7th and 15th centuries, though its exact origins remain unclear (Chouvy, 2008). Moroccan farmers traditionally cultivated a locally adapted variety of cannabis known as 'Beldiya'. However, cannabis cultivation has recently evolved towards the modernization of farming techniques and the adoption of new, high-yielding hybrid varieties as such as 'Mexicana' and 'Critical Plus' are distributed throughout the traditional growing areas in the central Rif (Clarke, 1998; Chouvy and Macfarlane, 2018; El Bakali et al., 2022).

Seed germination is a complex physiological process, regulated by numerous signals and influenced by both internal and external factors (Miransari and Smith, 2014). Internal factors include seed dormancy and stored nutrients, while external factors such as water, temperature, oxygen, light, and relative humidity play a crucial role (Bhardwaj et al., 2014; Savaedi et al., 2019). Among these, temperature stands out as the most significant environmental factor governing plant growth and development

(Koger et al., 2004; Ghaderi et al., 2008). The optimal temperature for achieving the highest germination and emergence rates varies across different crops (Motsa et al., 2015). A clear understanding of how crop seeds respond to temperature is essential, as it helps identify their tolerance to both low and high temperatures, as well as the climatic conditions necessary for successful germination and establishment. This knowledge also aids in developing models to predict crop development processes (Ghaderi et al., 2008).

Some research studies showed that the number of germinated seeds of hemp, including wild hemp increases linearly as the temperature rises to an optimal level and then decreases linearly as the temperature exceeds the limit (Haney and Kutscheid, 1975; Hilhorst et al., 1998). Optimal germination temperatures generally range around 24°C, although this relatively high temperature is often associated with late planting (Gbèhounou et al., 2000). While seedling development is slow at lower temperatures, hemp seeds can still germinate at temperatures approaching 0°C.

When crops are healthy and resources such as water and nutrients are abundant, dry matter production is directly proportional to the amount of light intercepted by the foliage (Monteith, 1977; Meijer et al., 1995). Baseline temperatures can be estimated by growing plants in controlled environments across a range of temperatures and analyzing their development or growth rate in relation to temperature (Adkins et al., 2002; Alvarado and Bradford, 2002). This is achieved by regressing growth rate against temperature and extrapolating the linear relationship to determine where it intersects the temperature axis (Milford and Riley, 1980; Ong, 1983; Villalobos and Ritchie, 1992). Additionally, some researchers have utilized data from field crops exposed to varying temperatures due to differences in locations, years, and planting dates (Arnold, 1959; Angus et al., 1980; Hegarty et al., 1980).

Knowledge of how temperature influences the germination and emergence of *Cannabis sativa* cultivars in Morocco remains limited. Therefore, this study aimed to investigate the effect of temperature on the seed germination and emergence of five *Cannabis sativa* cultivars in Morocco: 'Avocat', 'Beldiya', 'Critical Plus', 'Khardala', and 'Mexicana'. The specific objectives of this research were to assess the impact of temperature on several agrobiological parameters, including final germination percentage (FGP), total germination time (TGT), mean germination time (MGT), mean daily germination (MDG), and precocity or latent life span (LLS).

Results

Germination of the seeds of cultivars

The results of germination rates (Table 1, Figure 1-a) indicates that Cannabis seeds do not exhibit dormancy, as all seeds harvested from the plants successfully germinated. However, germination rates differ between cultivars and across temperatures within the same cultivar. For example, seeds from the 'Beldiya' cultivar show the highest germination rate at 10°C, reaching 81.11%. In contrast, the 'Mexicana' cultivar demonstrates a moderate germination rate of 40.56% at 25°C, similar to the 40% germination rate observed for 'Avocat' seeds at 20°C. The 'Critical Plus' cultivar achieves a germination rate of 37.78% at 25°C, while 'Khardala' seeds exhibit the lowest germination rates, not exceeding 31% at 25°C. Overall, the data suggest that both temperature and cultivar significantly influence germination rates, with variations evident both within individual cultivars and between different cultivars at various temperatures (Figure 1a).

Final germination percentage for each cultivar

The analysis of our data (Table 1) reveals significant variability in germination rates across different temperatures and cultivars. For example, the 'Critical Plus' cultivar exhibits the slowest germination speeds at temperatures of 30°C, 25°C, and 20°C, with

values ranging from 0.40 to 0.94 days. Similarly, the 'Khardala' cultivar shows low germination speeds, ranging from 0.58 to 0.97 days. In contrast, the 'Beldiya' cultivar demonstrates the fastest germination speeds, with values between 0.80 and 2.76 days. The kinetics of germination directly correlates with germination speed; as the speed increases, the overall germination rate also rises.

Seeds germinate effectively under suitable conditions, transitioning from a dormant to an active state. The median germination rates vary among cultivars: 'Khardala' at 27.56%, 'Mexicana' at 30.33%, 'Critical Plus' at 34.00%, 'Avocat' at 34.22%, and 'Beldiya' at 72.44%. This variation in median germination rates indicates considerable differences in germination efficiency both within and between cultivars.

Kinetics

Cumulative germination curves of seeds of different cultivars

Based on laboratory experiments and the methodology outlined in the Materials and Methods section, Cannabis seeds do not exhibit dormancy. Optimal germination occurs at around 25°C in the presence of light. When tested in the dark across a range of five temperatures (30°C, 25°C, 20°C, 15°C, and 10°C), the seeds failed to germinate (Figure 2).

Kinetics of seed germination of the studied cultivars at 30 °C

The cumulative germination curves follow a sigmoidal pattern, characterized by an initial exponential phase with a steep slope, followed by a plateau. This trend allows us to classify the cultivars into two distinct groups. The first group, represented by 'Beldiya', is notable for its rapid germination, achieving a rate of 64.44%. The second group includes 'Mexicana', 'Khardala', 'Avocat', and 'Critical Plus', which exhibit a gentler slope and have germination rates ranging from 17.78% to 33.89%.

The data also shows that all seed samples from the studied cultivars begin germinating on the first day of the experiment, though at different rates. Germination ceases by the fourth day (Figure 2a).

Kinetics of seed germination of the studied cultivars at 25 °C

The cumulative germination curves exhibit a sigmoid shape, comprising three distinct phases. The first phase, from day 0 to day 1, shows a flat slope, representing the latency period, which varies slightly among cultivars. The second phase, occurring between days 1 and 2, is marked by a steep slope in some cultivars and a more gradual incline in others, indicating the active germination phase for the majority of seeds. The third phase, from days 2 to 4, has an almost flat slope, corresponding to the final stages of germination for the remaining seeds.

Based on germination kinetics, two distinct groups emerge. The first group, represented by the cultivar 'Beldiya', exhibits a high germination rate of 77.78%. The second group includes 'Mexicana', 'Khardala', 'Avocat', and 'Critical Plus', with germination rates ranging from 30.56% to 40.56%. As shown in Figure 2b, all seeds from the different cultivars begin germinating by the first day of the experiment, albeit at different rates, with germination ceasing by the fourth day.

Kinetics of seed germination of the studied cultivars at 20 °C

The cumulative germination curves follow a sigmoidal pattern, consisting of three distinct phases. The first phase, from 0 to 0.5 days, has a flat slope, representing the latency period, which varies between cultivars. The second phase, spanning 0.5 to 1, 2, or 3 days, is marked by a steep slope for some cultivars and a more gradual incline for others, reflecting the active germination phase for the majority of seeds. The third phase, from 3 to 4 days, shows a nearly

Table 1. Germination parameters measured in five hemp cultivars.

Cultivars	Temperature	FGP (%)	MGT (Day)	MDG	TGT (Day)	LLS (Day)
'Beldiya'	T = 30°C	64.44	1.41	16.11	3.33	1.00
	T = 25°C	77.78	0.86	19.44	3.33	1.00
	T = 20°C	73.33	0.80	18.33	3.67	1.33
	T = 15°C	65.56	1.14	13.11	4.83	1.00
	T = 10°C	81.11	2.76	8.11	9.00	2.67
'Mexicana'	T = 30°C	23.33	0.70	5.83	3.17	1.00
	T = 25°C	40.56	0.68	10.14	3.17	1.50
	T = 20°C	39.44	1.07	9.86	3.67	1.83
	T = 15°C	23.33	1.55	4.67	4.67	3.67
	T = 10°C	25.00	2.70	2.50	8.83	5.00
'Khardala'	T = 30°C	17.78	0.58	4.44	2.33	1.00
	T = 25°C	30.56	0.73	7.64	3.00	1.17
	T = 20°C	30.00	0.97	7.50	3.67	1.50
	T = 15°C	30.56	1.25	6.11	4.50	1.50
	T = 10°C	28.89	2.14	2.89	7.67	3.00
'Avocat'	T = 30°C	31.11	0.59	7.78	3.00	1.00
	T = 25°C	36.11	0.67	9.03	3.00	1.17
	T = 20°C	40.00	1.03	10.00	3.50	1.17
	T = 15°C	30.00	1.58	6.00	4.67	1.83
	T = 10°C	33.89	2.17	3.39	7.83	3.33
'Critical Plus'	T = 30°C	33.89	0.40	8.47	2.00	1.00
	T = 25°C	37.78	0.55	9.44	3.00	1.00
	T = 20°C	36.67	0.94	9.17	3.33	1.00
	T = 15°C	34.44	1.34	6.89	4.33	1.67
	T = 10°C	27.22	2.35	2.72	8.17	3.83

FGP: Final germination percentage; MGT: Mean germination time; MDG: Mean daily germination; TGT: Total germination time; LLS: Latent life span.

flat slope, indicating the final germination stage for the remaining seeds.

Based on germination kinetics, two distinct groups can be identified. The first group consists of the cultivar 'Beldiya', which exhibits a high germination rate of 73.33%. The second group includes 'Mexicana', 'Khardala', 'Avocat', and 'Critical Plus', with germination rates ranging from 30.00% to 40.00%. As illustrated in Figure 2c, all seeds across the cultivars begin germinating by the first day of the experiment, albeit at different rates, with germination concluding after the fourth day.

Kinetics of seed germination of the studied cultivars at 15 °C

The cumulative germination curves display a sigmoidal shape characterized by three distinct phases. The first phase, lasting from 0 to 1 day, shows a zero slope, indicating the latency period, which varies slightly among cultivars. The second phase, spanning from 2 to 4 days, features a steep slope for some cultivars and a more gradual incline for others, representing the primary germination phase for most seeds. The third phase, occurring from 4 to 5 days, exhibits an almost flat slope, signifying the final stages of germination for the remaining seeds.

The data reveal two distinct groups based on germination kinetics. The first group includes the cultivar 'Beldiya', which has a high germination rate of 65.56%. The second group consists of 'Mexicana', 'Khardala', 'Avocat', and 'Critical Plus', with germination rates ranging from 23.33% to 34.44%. Figure 2d shows that all seeds from the various cultivars start to germinate by the second day of the experiment, although they do so at different rates. Germination stops after the fifth day.

Kinetics of seed germination of the studied cultivars at 10 °C

The cumulative germination curves are sigmoidal in shape and consist of three distinct phases. The first phase, spanning from 0 to 2 days, displays a zero slope and corresponds to the latency period, which varies slightly among cultivars. The second phase, from 2 to 6 or 8 days, features a steep slope for some cultivars and a more

Table 2. Correlation coefficients among the germination parameters in five hemp cultivars grown in central northern Morocco. Matrix correlation was carried between measured parameters.

FGP	T30	T25	T20	T15	T10
T30	-	0.023 [*]	0.145	0.897	0.691
T25		-	0.957	0.220	0.434
T20			-	0.628	0.855
T15				-	0.995
T10					-
MGT	T30	T25	T20	T15	T10
T30	-	1.0000	0.9307	0.2659	0.0120 [*]
T25		-	0.9307	0.2659	0.0120 [*]
T20			-	0.7514	0.1152
T15				-	0.7514
T10					-
MDG	T30	T25	T20	T15	T10
T30	-	0.266	0.628	0.975	0.373
T25		-	0.975	0.070	0.001 [*]
T20			-	0.266	0.012 [*]
T15				-	0.751
T10					-
TGT	T30	T25	T20	T15	T10
T30	-	0.995	0.434	0.054	0.002 [*]
T25		-	0.691	0.145	0.009 [*]
T20			-	0.855	0.266
T15				-	0.855
T10					-
LLS	T30	T25	T20	T15	T10
T30	-	0.957	0.497	0.220	0.003 [*]
T25		-	0.897	0.628	0.031 [*]
T20			-	0.987	0.266
T15				-	0.562
T10					-

*Correlation is significant at the 0.05 level (two-tailed).

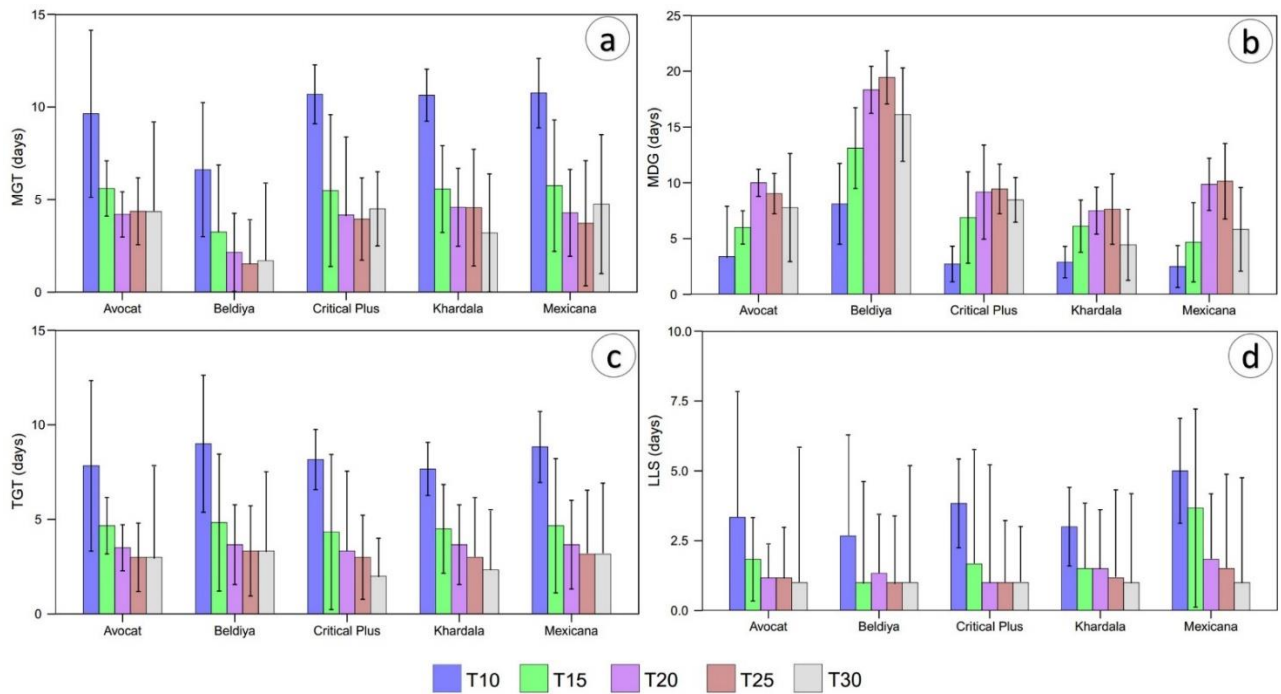


Fig. 1. The effect of temperature on four germination variables in five hemp cultivars grown in central-northern Morocco, assessed at five temperatures: 10°C, 15°C, 20°C, 25°C, and 30°C. (a) Mean Germination Time (MGT); (b) Mean Daily Germination (MDG); (c) Total Germination Time (TGT); (d) Latent Life Span (LLS).

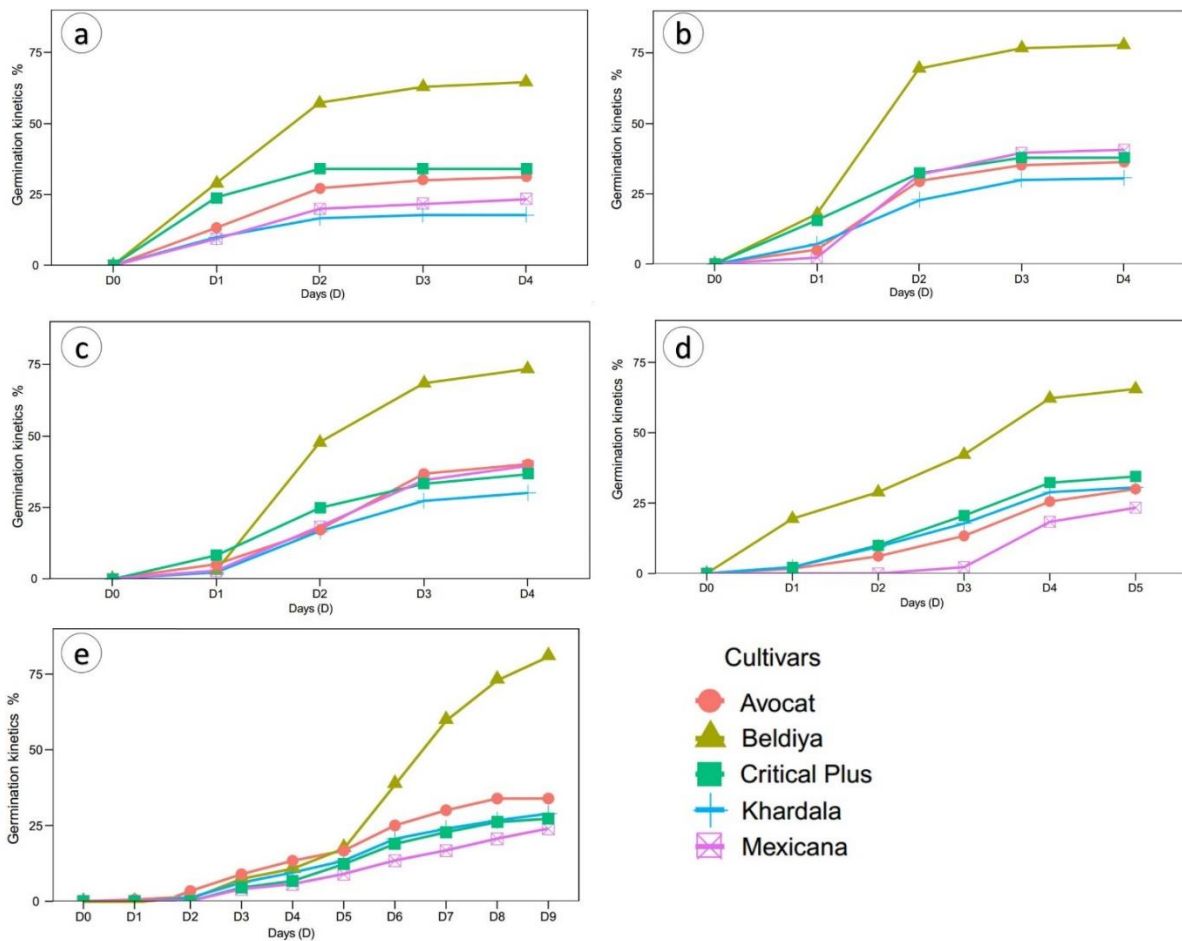


Fig. 2. Germination kinetics curves of seeds from five *Cannabis sativa* cultivars at the following temperatures: (a) 30°C; (b) 25°C; (c) 20°C; (d) 15°C; and (e) 10°C.

gradual incline for others, representing the primary germination phase for most seeds. The third phase, from 8 to 10 days, exhibits a nearly flat slope, indicating the final stages of germination for the remaining seeds.

The data reveal two distinct groups based on germination kinetics. The first group includes the cultivar 'Beldiya', which has a high germination rate of 81.11%. The second group comprises 'Mexicana', 'Khardala', 'Avocat', and 'Critical Plus', with germination rates ranging from 25.00% to 33.89%. Figure 2e illustrates that all seeds from the different cultivars begin to germinate by the second day of the experiment, though at varying rates. Germination ceases after the tenth day.

Mean germination time (MGT)

The results shown in Table 1 indicate significant differences in mean germination time among various temperatures and cultivars. Notably, temperatures of 20°C, 25°C, and 30°C produce the shortest mean germination times, averaging 0.96 days, 0.70 days, and 0.74 days, respectively. In contrast, the mean germination time at 10°C is considerably longer, averaging 2.42 days. Meanwhile, the temperature of 15°C yields mean germination times that do not exceed 1.37 days (Figure 1a).

Among the cultivars, 'Beldiya' and 'Mexicana' (both in the first group) exhibit the shortest mean germination times and the highest germination rates. Conversely, cultivars in the second group demonstrate lower average germination rates compared to these two leading cultivars.

Mean daily germination (MDG)

The results presented in Table 2 and the accompanying graph demonstrate significant differences in germination times among cultivars. The cultivar 'Beldiya' has the longest average germination time at approximately 15.02 days, while 'Khardala' has the shortest at 5.72 days. The other cultivars fall within this range, displaying average values between these two extremes (Figure 1b).

The data also reveal considerable variation in germination times at different temperatures. Cultivars in the first group generally exhibit higher daily average germination rates compared to those in other groups. Specifically, temperatures of 25°C and 20°C correspond to the highest average germination rates. In contrast, temperatures of 15°C and 30°C produce moderate average values, while 10°C results in the lowest average germination rate. These findings indicate that higher temperatures typically accelerate germination, whereas lower temperatures hinder the process.

Total germination time (TGT)

Table 2 shows significant variation in total germination time (TGT) among different cultivars and across temperatures within the same cultivar. The cultivars 'Beldiya' and 'Mexicana' are particularly long-lived, exhibiting the longest average germination durations at 10°C. In contrast, the cultivar 'Critical Plus' has the shortest durations at higher temperatures, specifically at 25°C and 30°C (Figure 1c).

At 10°C, the average germination duration is the longest, followed by 15°C. Other temperatures result in shorter durations, with the germination time at 10°C being longer than that at the higher temperatures of 25°C and 30°C. As the temperature increases, both germination duration and germination rate decline.

The precocity or latent life span (LLS)

This parameter exhibits significant variation with temperature. Table 1 indicates that at temperatures of 30°C, 25°C, and 20°C, values range from 1 to 2 days. In contrast, at 10°C, the range broadens to 1 to 8 days. This demonstrates a considerable variation

in latent life among cultivars, with durations spanning from 1 to 8 days.

The figure below further illustrates that cultivars in the first group have shorter latent periods compared to those in the second group. This suggests that as the latent period increases, the germination rate tends to decrease.

Correlation analysis

The correlation analysis reveals strong positive correlations among various germination parameters (Table 2). This suggests that cultivars with higher germination rates tend to have shorter times to germination; in other words, a higher germination rate is associated with a quicker time to achieve it. However, the data indicates no significant correlation between the final germination percentage (FGP) and lower temperatures. This implies that low temperatures do not substantially affect the total germination percentage (FG) of the seeds. Additionally, the correlation coefficients for total germination time (TGT), precocity or latent life span (LLS), and mean germination time (MGT) in relation to higher temperatures show no clear relationship between these parameters and elevated temperatures.

Discussion

In the laboratory germination tests conducted under controlled conditions, all seeds exhibited germination capability, indicating that there is no dormancy in Cannabis seeds. The seeds used in this study were collected from the Fifi region (Northwestern Morocco). To analyze the impact of temperature on various agrobiological parameters, a two-sided multiple pair comparison test utilizing the Nemenyi procedure was employed. This analysis categorized the temperature range into three distinct groups: a high-effect group, a low-effect group, and a moderate-effect group. The analysis of germination test results under varying temperature conditions revealed differing behaviors among cultivars and temperatures. The temperature of 25°C had the most significant positive effect on the final germination percentage (FGP), while 30°C had the least effect. Other temperatures showed moderate effects.

These findings align with previous research indicating that the optimal temperatures for Cannabis seed germination generally range around 24°C (Ceapoiu, 1958; Small and Brookes, 2012). Studies have also shown that hemp seed germination rates and percentages are highest between 19°C and 30°C (Geneve et al., 2022; Varga et al., 2022), with the minimum germination temperature reported as 0°C (Van der Werf et al., 1995; Pospíšil, 2013). Typically, germination occurs within three to seven days (Clarke, 1997).

Interestingly, the observation of faster germination rates at temperatures above the optimum is atypical, as most species generally exhibit slower germination times at suboptimal temperatures (Bradford, 2002; Patanè et al., 2023). However, in some species, germination rates can plateau at super-optimal temperatures (Orozco-Segovia et al., 1996; Park et al., 2022).

The impact of temperature on mean germination time (MGT) decreases as the temperature increases. The Multiple Comparison test identified 10°C as having the strongest effect, while temperatures of 20°C, 25°C, and 30°C exhibited relatively weaker effects. The temperature of 15°C had a moderate impact. According to Bierhuizen (1973), Angus et al. (1980), and Qin et al. (2014), base temperatures for most temperate crops range between 0°C and 4°C, while those for crops adapted to subtropical and tropical regions fall between 8°C and 15°C.

Total germination time (TGT) and latent life span (LLS) exhibit similar temperature-dependent behavior, with both values inversely proportional to temperature. The most significant effect

Table 3. Main characteristics of the *C. sativa* cultivars studied.

Cultivar	'Avocat'	'Beldiya'	'Critical Plus'	'Khardala'	'Mexicana'
Origin	Unknown	Morocco	Netherlands	Unknown	Unknown
Sexuality	Dioecious	Dioecious	Dioecious	Dioecious	Dioecious
Mean plant height (cm)	121.48	95.92	111.98	92.25	133.56
Sowing time	April-May	Mars-April	April-May	April-May	April-May
Harvest time	September-October	July-August	September-October	September-October	September-October
Sowing to flowering (months)	3-4	2-3	3-4	3-4	3-4
Sowing to harvest (months)	6	5	6	6	6
Voucher	BAH 3065, BAH 3069, BAH 3071	BAH 3081, BAH 3083, BAH 3084	BAH 3088, BAH 3091	BAH 3111, BAH 3113	BAH 3122, BAH 3125

was observed at 10°C, while 25°C and 30°C had weaker impacts, and 15°C and 20°C had moderate effects. Tamm (1933) noted that hemp seeds require a specific photoperiod for germination and emergence. Additional reports on temperature and humidity for maintaining hemp seed germination, although less detailed, were provided by Laskos (1970) and Demkin and Romanenko (1978). Daily mean germination (MDG) also follows a similar temperature pattern. The strongest effects were seen at 20°C and 25°C, with moderate effects at 15°C and 30°C, and the weakest effect at 10°C. Parihar et al. (2014) found no significant differences in final germination percentages at 20°C, 25°C, and 30°C. However, they reported no germination at 15°C and thermoinhibition at 30°C and 35°C (Patanè et al., 2023).

In summary, lower temperatures tend to prolong mean germination time (MGT), TGT, and LLS, while higher temperatures enhance MDG. Slightly elevated temperatures are beneficial for achieving higher final germination percentages (FGP).

Materials and Methods

Plant material and seeds collection

This study focused on five widely cultivated *Cannabis sativa* L. cultivars from the Rif Mountains: 'Beldiya', a Moroccan cultivar adapted to the region's specific environmental conditions, and 'Avocat', 'Critical Plus', 'Khardala', and 'Mexicana', which were introduced for their high yields and elevated THC levels. These cultivars are named according to local designations. Seeds for these cultivars were sourced from farmers in the Fifi region (Bab Taza) in Chefchaouen Province, located in northwest Morocco. Detailed information on these cultivars is provided in Table 3.

Sampling was conducted on plants harvested a year prior to the germination tests in the Fifi region (northwestern Morocco), approximately 25 km from Chefchaouen (35°10'17"N 5°16'11"W, 600 m.a.s.l.). This area of central-northern Morocco has a Mediterranean climate, with an average annual temperature of 15.3°C and about 878 millimeters of annual precipitation.

During the field trips, we collected only the seeds of interest, ensuring they were uniformly brown and healthy. We gathered half a kilogram of seeds from each cultivar, a sufficient quantity for the germination experiments. After collection, the seeds were stored in small paper bags to protect them from loss, insects, and moisture. The seeds from five commercial *C. sativa* cultivars: 'Avocat', 'Beldiya', 'Critical Plus', 'Khardala', and 'Mexicana', were then stored in a dry environment at room temperature.

Seed germination

After sample collection, germination tests for Cannabis seeds from various cultivars were conducted at the Applied Botany Laboratory, Faculty of Science, Tetouan. The seeds were stored under optimal temperature and humidity conditions, with the bags remaining sealed until needed for testing. To remove surface

pathogens, seeds were initially treated with a 2% sodium hypochlorite solution for 1 minute, as outlined by Kassaoui et al. (2009). In our procedure, surface contamination was further addressed by washing the seeds with 70% ethanol, followed by a thorough rinse with sterilized distilled water.

The germination test for the collected seeds was carried out using Petri dishes lined with three layers of filter paper moistened with distilled water, following the method described by Lachhab et al. (2013). Seeds were inspected under a magnifying glass, and germination was defined as the emergence of the radicle through the seed coat (Chergui et al., 2024).

To determine the optimal germination temperature, each of the five cultivars was tested at five different temperatures ranging from 10°C to 30°C (Berka and Harfouche, 2001; Small and Brookes, 2012). Each temperature condition was tested with six replicates per cultivar, with 30 seeds per Petri dish. The dishes, containing two layers of filter paper soaked with 10 ml of distilled water, were incubated in the dark at the specified temperatures (Figure 3).

Measured parameters

After the germination of the seeds, the following parameters could be performed:

- **Final germination percentage or germination percentage (FGP):** expressed as the ratio of the number of germinated seeds to the total number of seeds (Lachiheb et al., 2004; Vernay et al., 2009; Alaoui et al., 2013).
- **Germination kinetics:** the number of germinated seeds is counted daily until germination stabilizes (Alaoui et al., 2013).
- **Mean germination time (MGT):** calculated by the following formula: $MGT = \sum (n_i \times t_i) / N$, Where n_i is the number of seeds germinated at time t_i and N is the number of germinated seeds at the end of the trial (Hartmann et al., 1990; Berka and Harfouche, 2001).
- **Mean Daily Germination (MDG):** according to Osborne and Nercer (1993), MDG is the ratio of the final germination rate to the number of days it took the last seed to germinate (Alaoui et al., 2013).
- **Total germination time (TGT):** the number of days for all the seeds to be germinated.
- **Precocity or latent life span (LLS):** the number of days that seeds require before they begin to germinate (the latent phase) or the time after which the first germination of the experimental batch occurs (Benmahioul et al., 2010).

Statistical analysis

The raw data from the germination tests were statistically analyzed to evaluate the agrobiological parameters. Descriptive statistics, including mean, variance, standard deviation, and confidence intervals, were calculated for the six replicates of each cultivar at each temperature. Further analysis involved inferential statistics, specifically the two-tailed Multiple Pairwise Comparisons test

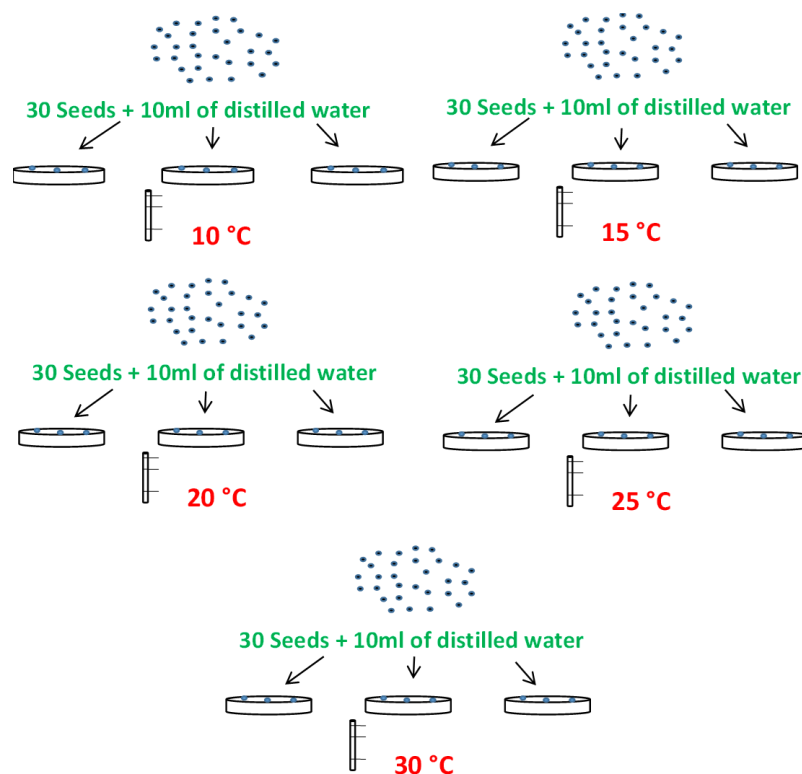


Fig. 3. Experimental procedure for seed germination tests of the studied Cannabis cultivars.

using the Nemenyi procedure. Data visualization was performed using R software (R Core Team, 2020) and XLSTAT 2021 for graphical representation.

Conclusion

The germination study revealed that none of the cultivars exhibited dormancy, with final germination percentages ranging from moderate to high. Analysis of germination kinetics identified two distinct groups: one group with final germination percentages between 23.33% and 40.56%, and another group with higher percentages ranging from 64.44% to 81.11%. Temperature analysis demonstrated a strong positive correlation between final germination percentage (FGP) and higher temperatures, indicating that elevated temperatures promote higher germination rates. Regarding other germination parameters, mean germination time (MGT), mean daily germination (MDG), total germination time (TGT), and latency or latent life span (LLS) significant variation was observed both between cultivars and in response to temperature changes within each cultivar. This variation distinguishes two groups: the first group, which includes 'Beldiya' and 'Mexicana', shows higher values for MGT, MDG, FGP, and TGT, while the second group, comprising 'Khardala', 'Avocat', and 'Critical Plus', has lower values for these parameters. However, in terms of LLS, the second group demonstrates higher values compared to the first.

Ethic statement

Research did not include any human subjects and animal experiments

CRedit authorship contribution statement

Ismail El Bakali: Conceptualization, Data collection, Methodology, Investigation, Writing - original draft. **Soufian Chakkour:** Writing - review & editing, Statistical analysis, Visualization. **Samir El Bakali:** Data collection, Writing - original draft. **Mohamed Kadiri:** Identified the botanical taxa,

Methodology, Writing - original draft. **Abderrahmane Merzouki:** Conceptualization, Methodology, Supervision, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data Availability

Data will be made available on request.

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