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Germination Response of Different Castor Bean Genotypes to Temperature for Early and Late Sowing Adaptation in the Mediterranean Regions

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Abstract: Germination of castor seeds of seven dwarf hybrid genotypes, compared to a ‘Local’ genotype, selected from a Tunisian population by the University of Catania well adapted to the Mediterranean environment, were studied at six different temperatures (8, 12, 16, 25, 32, and 40 °C). The results indicate that the optimal temperature (25 °C) and near-optimal temperature (32 °C) are the best temperatures for ensuring castor germination (final germination percentage (FGP) $\geq$ 82.81%). Furthermore, these temperatures positively influenced the vigour index (VI) and the radicle elongation. At a temperature of 8 °C, no germination occurred, while temperatures of 12 and 40 °C negatively affected the seed germination, which, in some genotypes, was null or negligible (<21.25%). A temperature of 16 °C allowed good results to be reached for the FGP and the other considered parameters. Overall, the dwarf hybrids performed better at high temperatures than at low temperatures, thus, making them suitable for late sowings, with the exception of the genotype ‘C1020’, which resulted the best performance at 16 and 40 °C, being suitable for both early and late sowings. On the other hand, the ‘Local’ castor genotype, being the best-performing genotype at 12 and 16 °C, and the most tolerant to low temperature (base temperature (Tb) 12.1 °C), could be used in the early sowing in spring.

Keywords: cardinal temperatures; dwarf hybrids; seed germination; seed vigour; synchrony

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1. Introduction

Germination is a key stage in the establishment of plants. Germination consists of the activation of the metabolic mechanisms of the seed, which leads to the birth of a new seedling. To obtain the germination of a mature seed, three conditions should be satisfied: (1) the seed must be vital; (2) seed environmental requirements must be appropriate; and (3) any form of primary dormancy must be overcome.

The germination process begins with the absorption of water by the dry seed and ends with the elongation of the embryo [1]. During the imbibition, the seed is not uniformly moistened and this stage can be divided into two steps: the first one comprehends the hydration of the outer part of the seed, while the second includes the hydration of the inner part, the activation of metabolic processes, and, ultimately, the radicle extrusion through the structures surrounding the embryo [2]. Environmental factors such as soil moisture, temperature, oxygen, light, and pH influence seed germination in wild and cultivated plants [3]. In particular, optimal rates of seed germination and, consequently, plant establishment, are the first conditions required to achieve adequate levels of crop productivity, even under harsh weather conditions [4]. Therefore, it is important to analyse the influence of biotic and abiotic conditions that can maximize or limit seed germination in the field and the following crop establishment [5].

Castor bean (*Ricinus communis* L.) is a plant belonging to the Euphorbiaceae family. It is a non-edible vegetable oil crop with lots of applications in the industrial and medical sectors [6]. Different from other oilseed crops, the interest toward this plant has increased exponentially in recent years due to its high seed oil content (around 35–65%) and a high percentage of ricinoleic acid (85–90%). The latter, in particular, has lately been re-evaluated for its wide range of industrial applications [7].

The chance to use marginal lands for its cultivation, and therefore avoid competition with food crops in the use of more valuable lands, may facilitate castor's exploitation [8,9]. Despite the multiple purposes that castor oil can satisfy, its main use remains the production of fuels such as biodiesel, to conform with the agricultural policies of the Agenda 2030 to produce green energy, replacing fossil sources [10].

As a crop native to Africa, castor is well adapted to tropical and subtropical climates. In the Mediterranean environment, its adaptability expression and cultivation requirements are easily satisfied. Studies confirm that castor needs a range of temperatures between 25 and 30 °C during germination [11] and an amount of water of 400 L per kg of seeds produced [12].

In the semiarid regions of the Mediterranean Basin, the assessment of seed germination response to temperature is important in castor, to predict the seed performance under different thermal conditions that the plant may experience during the initial stages of the growing season. This is particularly true when early sowings are required, to make better use of the water stored in the soil during the rainy period. Therefore, it is also important to identify the thermal thresholds in the seed germination of castor, which may limit the adoption of early or late sowing. On the other hand, the identification of the maximum temperature for germination in castor is also important, to fix the time threshold for late sowing, when castor follows a winter crop within cropping systems. Therefore, this study aimed to evaluate and compare the seed germination characteristics of different genotypes of castor under different thermal conditions. The main purpose of this research was to define the limits in the adoption of early or late sowing in these genotypes, with the final goal of better exploiting resources (water, soil, etc.) from the perspective of environmental sustainability.

2. Materials and Methods

The experiment was conducted in a laboratory of the University of Catania studying two experimental factors: genotype and temperature. There were eight genotypes: seven dwarf hybrids of castor bean (*Ricinus communis* L.), provided by Kaiima Company (Campinas—São Paulo, Brasil), compared to a 'Local' genotype of castor, mass-selected for adaptation to the Mediterranean environment from a wild Tunisian population at the Department of Agriculture, Food and Environment (Di3A) of the University of Catania, used as control.

The seeds of the genotypes were assessed for germination traits at six constant temperatures: 8, 12, 16, 25, 32, and 40 °C, with 25 °C considered as the control, being reported as the optimal temperature for seed germination in castor according to ISTA (International Seed Testing Association) [13].

2.1. Seed Material

The seeds were open-field-produced between April and November 2022 at the experimental farm of the University of Catania (37°24′31″ N; 15°3′33″ E, Italy).

The experiment was conducted on seeds harvested in November 2022, stored in paper bags, and kept at room temperature (10–20 °C) until use. Seeds were surface-sterilized with 1% sodium hypochlorite solution (NaClO) (Sigma-Aldrich s.r.l., Milano, Italy) for 3 min, rinsed in distilled water, and then dried at room temperature for 24 h, before germination tests. Before the tests, the seed weight (g) was measured in all genotypes, in eight replicates of 100 seeds each. Seed moisture contents ranged between 3.5 and 4%.

2.2. Germination Tests

For each genotype and temperature, four replicates of 20 seeds each were placed in Petri dishes (Ø 120 mm) containing two filter papers—one above and one below the seeds—to allow complete seed hydration. Each filter was moistened with 5 mL of distilled water. Petri dishes were hermetically sealed with Parafilm to avoid evaporation and randomly placed in a thermostatically controlled (±1 °C) incubator, in dark conditions, at one of the above-mentioned temperatures. Seeds were considered germinated when the testa broke near the caruncle and the radicle emerged from the seed (at least 2 mm long). The Petri dishes were checked daily for germination. Germination was scored until no further germination occurred for at least one week. At the lowest temperatures (8, 12, and 16 °C), the maximum period of observation was extended to 30 days.

The germination tests were conducted between the end of 2022 and the beginning of 2023.

2.3. Radicle Length Measurements

The length of the radicle was measured in seeds of all genotypes at all temperatures. For this purpose, five seeds per replicate were randomly chosen from those first germinated, on the fourth day from the recorded start of germination (i.e., first radicle appearance) (Figure 1). In the case of germination being lower than 5 seeds, only those germinated were measured. Seeds were then photographed to document the radicle length at different temperatures.

Figure 1. Germination of castor seeds, photographed on the fourth day from the recorded start of germination using an iPhone X smartphone.

Photos were taken using an iPhone X smartphone. Scanned images were then analysed using ImageJ software, a Java image open-source program used for processing digital images, developed at the National Institutes of Health (NIH) [14]. Because the program requires a minimum input of information, it is necessary to indicate the correct metric in the desired image. This can be conducted by including an object whose dimensions are known in the picture. In the current work, a ruler was considered for the calibration. After setting the reference measurement, the radicle length in each seed was measured simply using the cursor.

2.4. Calculations and Data Analysis

At the end of the germination tests, the parameters reported in Table 1 were calculated.

Table 1. Germination parameters calculated at the end of the tests.

Parameters	Symbol	Unit	Formula	Explanation	References
Final germination percentage	FGP	%	FGP = GN/SN × 100	GN = total number of seeds germinated; SN = total number of seeds tested	[15,16]
Mean germination time	MGT	Day	$\text{MGT} = \sum \left(\frac{ni \times di}{n}\right)$	ni = number of seeds germinated on day i; d is the incubation period in days; n the total number of germinated seeds.	[15]
Vigour index	VI	-	VI = FGP × radicle length	-	[17]
Synchrony of germination	Z	-	$Z = \sum_{i=1}^{n} Cn_{i;2} / \text{N}$	C_ni; 2 = $n_i(n_i - 1)/2$ and N = $\sum n_i (\sum n_i - 1)/2$, where C_ni; 2 is the combination of seeds germinated at the ith time, two together, and n_i is the number of seeds germinated at time i.	[18,19]

Data of the 100-seed weight were analysed using one-way ANOVA. Data of final germination percentage (FGP; previously arcsine transformed), mean germination time (MGT), radicle length, vigour index (VI), and data at 16, 25, and 32 °C for synchrony of germination (Z) were checked for normality and homogeneity of variances and statistically analysed via factorial two-way ANOVA. The data were processed using CoStat version 6.003 (CoHort Software), considering the genotype and the germination temperature as fixed factors. When "F" ratios were significant, means were separated using Tukey's test ($p < 0.05$). Data of germination occurred at 8 °C and are not shown, because no germination occurred at this temperature, as for data of Z at 12 and 40 °C, because low levels of synchronization were reported for some replicates.

The time course of the cumulative values of seed germination was described using a nonlinear iterative regression method (SIGMAPLOT® 9.0 software, Systat Software Inc., San Jose, CA, USA) using a sigmoidal model with three parameters.

$$y = \frac{a}{1 + \left(\frac{x}{x_b}\right) b}$$

where a is the maximal value of y (maximum germination), x is the time (days) after seed imbibition, x_0 is the time (days) to reach 50% of maximal germination, and b is a fitting parameter of the curve. The x value on the curve corresponds to 50% germination (y value of the curve) and was assumed as theoretical time to 50% germination or t_{50} (days).

The data set of germination rates of 50% germination fraction ($1/t_{50}$ or GR_{50}) of the seed population analysed at 16, 25, and 32° C, resulting from the germination time course, was plotted against T, separately for each cultivars; due to the low level of germination data at 12 and 40 °C, these were not considered in the Tb calculation. The theoretical minimum or base temperature (Tb) was calculated by the linear regression of GR_{50} vs. T, at which seed germination of each cultivar is reduced to 50%. The slope b of the regression line is the germination rate with a decreasing temperature (the higher the b, the faster the germination with the temperature increase). The abscissa intercept is an estimate of the theoretical minimum temperature of germination (Tb) [20,21]. The thermal time (θ_T) needed to achieve 50% germination ($\theta_{T(50)}$) at the temperatures evaluated was calculated according to the following equation:

$$(\theta_{T(50)}) = (T - T\text{b}) \times t_{50}$$

where $\theta_{T(50)}$ = thermal time needed for 50% germination (°Cd), T = actual germination temperature (°C, constant in controlled environment), Tb = base germination temperature, t_{50} = the time to 50% germination (median response time) [20,21].

To compare both methods of $\theta_{T(50)}$ calculation, thermal time was also calculated as the inverse of the slope b of the regression line used for Tb estimation.

3. Results

3.1. The 100-Seed Weight

The values of the 100-seed weight measured for the eight genotypes are reported in Table 2. According to the ANOVA, the weight was significantly affected by the genotype, ranging from 29.88 g (in 'C1008′) to 52.05 g (in 'C1013'). Seeds of the 'Local' castor exhibited a seed weight (33.88 g) lower than the average values of all genotypes.

Table 2. The 100-seed weight (mean ± SE) of 8 genotypes of castor. Values with different letters are significantly different at $p < 0.05$, according to Tukey's test.

Genotype	100-Seed Weight (g)
Local	33.88 ± 0.99 de
857	35.60 ± 0.37 bd
1008	29.88 ± 0.15 fh
1012	37.02 ± 0.07 b
1013	52.05 ± 0.07 a
1018	35.90 ± 1.20 cd
1019	35.78 ± 0.03 bc
1020	36.01 ± 0.07 bd
Average	37.01 ± 2.92

3.2. Cumulative Germination Time Course

Because no germination occurred at 8 °C, this temperature was not shown for all parameters. The cumulative seed germination time course of the eight genotypes of castor under different temperatures (12, 16, 25, 32, and 40 °C) is illustrated in Figure 2. The course is effectively described ($R^2 > 0.90$, Table 3) using a three-parameter sigmoidal function, whose trend reveals a short initial phase of low germination at optimal or near-optimal temperature (25 and 32 °C), followed by a rapid increase in germination, up to a maximum (*a* parameter of the curve). After that, germination remained constantly stable. In some genotypes, the initial phase of slow germination became negligible at 32 °C. At a high temperature (40 °C), seed germination started later than at an optimal temperature (25 °C) and proceeded poorly and slowly. Only in 'C1013′ and 'C1019′ did germination at 40 °C start earlier than at 25 °C, although germination at this temperature, in these two genotypes, was maintained lower than 50%. At the suboptimal temperature (12 °C), the start of germination was delayed to a great extent, and very low levels of germination were scored. At 16 °C, all the genotypes germinated, slower than at other temperatures, and only the 'Local' genotype approached 100%. The supraoptimal temperature (40 °C) enhanced the germination percentage and rate. Only in the 'Local' castor were the seeds able to germinate at 12 °C, approaching a maximum value (*a* parameter) that was even higher than that at 40 °C.

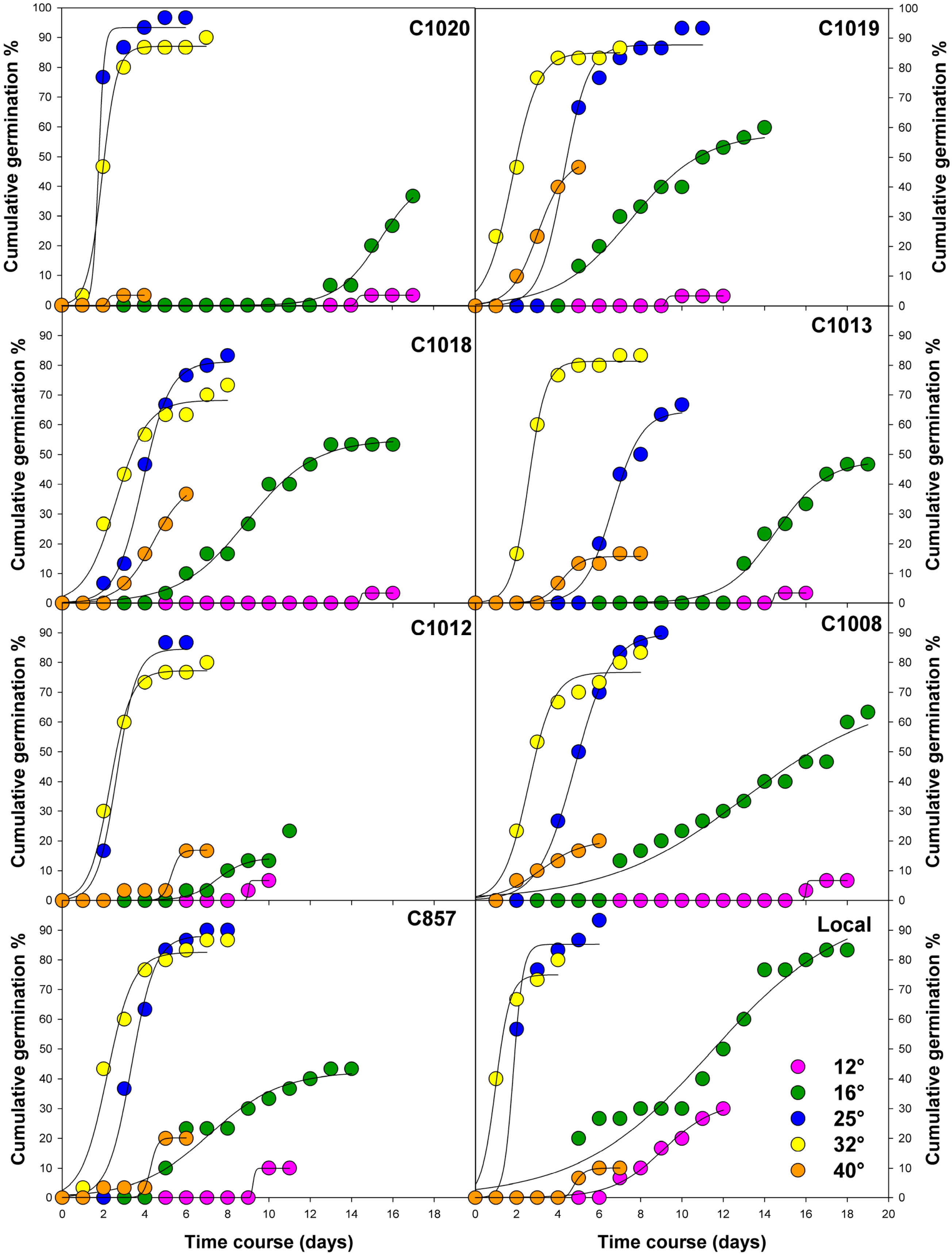

Figure 2. Cumulative germination time courses (solid curves) at different temperatures in 8 genotypes of castor. Symbols represent the observed daily percentages at 12, 16, 25, 32, and 40 °C vs. time.

Table 3. Values of parameters of the sigmoidal logistic function interpolating data of seed germination of 8 genotypes of castor at 12, 16, 25, 32, and 40 °C.

Genotype	T (°C)	a	R^2
Local	12	19.20	0.98
	16	32.20	0.99
	25	75.80	0.99
	32	75.00	0.98
	40	11.00	1.00
C857	12	11.00	1.00
	16	48.70	0.99
	25	88.00	0.98
	32	82.60	0.98
	40	20.00	0.95
C1008	12	6.60	1.00
	16	13.80	0.99
	25	89.80	0.99
	32	76.70	0.98
	40	20.30	0.97
C1012	12	6.66	1.00
	16	14.9	0.99
	25	84.50	0.99
	32	77.20	0.99
	40	1.68	0.93
C1013	12	3.33	1.00
	16	49.80	0.99
	25	64.40	0.98
	32	80.70	0.99
	40	15.00	0.98
C1018	12	3.59	1.00
	16	59.30	0.99
	25	81.20	0.99
	32	68.10	0.97
	40	41.10	0.99
C1019	12	3.33	1.00
	16	67.60	0.99
	25	74.70	0.98
	32	85.10	0.99
	40	49.00	0.99
C1020	12	3.59	1.00
	16	48.80	0.99
	25	92.20	0.99
	32	87.00	0.99
	40	3.33	1.00

3.3. Seed Germination under Controlled Temperatures

At the optimal temperature (25 °C), the final germination percentage (FGP) of seed germination reached 87.97% across the genotypes (Table 4). An FGP of 82.81% was also recorded at 32 °C, which was not significantly different. The FGP started to significantly decrease at 16 °C in the average for all the genotypes (63.72%) and significantly decreased down to 21.25% at 40 °C and 8.75% at 12 °C. The FGP, across temperatures, also changed with the genotype, although within a narrow range, being the highest in 'C1019′ (with a 64.75% FGP) and the lowest in 'C1013′ (43.25%). Good results were also shown for the 'Local' genotype, which was lower than the best with only a 5.50% difference.

Table 4. Mean effect and standard error (SE) of temperatures (T) and genotypes (G), and their interaction (TxG) on the final seed germination percentage (FGP), mean germination time (MGT), synchrony of germination (Z), radicle length, and vigour index (VI). Data of Z at 12 and 40 °C were excluded from the statistical analysis (see Section 2).

	FGP (%)	**MGT (day)**	**Synchrony of Germination (Z)**	**Radicle Length (cm)**	**VI**
Temperature (T)					
12 °C	8.75 ± 1.67 d	23.33 ± 2.21 a	-	0.60 ± 0.12 d	10.98 ± 2.68 d
16 °C	63.72 ± 3.77 b	12.05 ± 05.57 b	0.12 ± 0.03 c	1.50 ± 0.11 c	90.85 ± 8.20 c
25 °C	87.97 ± 2.31 a	4.65 ± 0.27 c	0.32 ± 0.03 a	1.96 ± 0.15 b	177.39 ± 14.92 b
32 °C	82.81 ± 1.50 a	3.14 ± 0.17 d	0.25 ± 0.02 b	4.54 ± 0.27 a	381. 21 ± 26.75 a
40 °C	21.25 ± 2.60 c	4.76 ± 0.31 cd	-	0.83 ± 0.04 d	16.47 ± 1.85 d
Genotype (G)					
C857	52.00 ± 7.73 ac	8.88 ± 1.61 bc	0.20 ± 0.03 b	1.77 ± 0.18 c	108.92 ± 22.84 ce
C1008	53.25 ± 7.56 ac	11.06 ± 2.31 a	0.15 ± 0.02 b	1.77 ± 0.33 c	125.24 ± 31.61 bd
C1012	48.00 ± 7.66 bc	8.25 ± 1.11 c	0.23 ± 0.05 b	2.36 ± 0.38 ab	159.33 ± 36.01 ac
C1013	43.25 ± 6.69 c	11.78 ± 1.50 a	0.19 ± 0.03 b	1.13 ± 0.24 d	71.30 ± 21.27 e
C1018	50.75 ± 6.74 ac	10.34 ± 1.31 ab	0.14 ± 0.02 b	1.22 ± 0.18 d	81.73 ± 15.70 de
C1019	64.75 ± 8.01 a	9.20 ± 1.25 bc	0.19 ± 0.02 b	1.88 ± 0.40 bc	162.33 ± 36.30 ab
C1020	51.95 ± 9.60 ac	11.20 ± 1.81 ab	0.47 ± 0.06 a	2.61 ± 0.51 a	207.10 ± 55.49 a
Local	59.25 ± 7.48 ab	5.99 ± 0.85 d	0.29 ± 0.02 ab	2.37 ± 0.37 ab	167.07 ± 37.01 ab
Significance					
T	***	***	***	***	***
G	**	***	***	***	***
T x G	*	***	ns	***	***

Values with the same letter are not significantly different at $p < 0.05$ level ($n = 4$). *, ** and *** indicate significance at $p < 0.05$, 0.01, and 0.001, respectively.

However, the significant T × G interaction indicated a different FGP response to temperature, depending on the genotype. Generally, the FGP at 32 °C matched that at 25 °C, in all genotypes (excluding 'C1013′, which was 66.25 and 83.75% at 25 and 32 °C, respectively) (Table 5). The genetic differences in the FGP became evident at extreme temperatures (12 and 40 °C). In particular, germination was significantly depressed at both 12 and 40 °C in all genotypes, and the FGP was reduced down to levels that, in most cases, were not different between the two temperatures. Only in the 'Local' genotype were the values of the FGP lower at 40 °C than at 12 °C (10.00 and 30.00%, respectively). In general, the genotypes that germinated at their best under optimal thermal conditions were 'C1019′ and 'C1020′, with final germination that approached 100%. Under the same conditions, 'C1013′ exhibited the lowest germination (66.25%). At 40 °C, the highest FGP (47.50%) corresponded to genotype 'C1019', which was also the genotype that had the highest germination at 16 °C (90.00%).

Table 5. Final germination percentage (FGP, %) in eight genotypes of castor at five temperatures (12, 16, 25, 32, 40 °C) ($n = 4$).

Temperature	**Genotype**							
(T)	**C857**	**C1008**	**C1012**	**C1013**	**C1018**	**C1019**	**C1020**	**Local**
12 °C	10.00 c B	10.00 b B	5.00 c B	3.75 c B	3.75 c B	3.75 c B	3.75 b B	30.00 b A
16 °C	53.75 ab	62.50 a	50.00 ab	46.25 b	56.25 ab	90.00 a	67.25 a	83.75 a
25 °C	90.00 a	90.00 a	86.25 a	66.25 a	83.75 a	97.50 a	97.50 a	92.50 a
32 °C	86.25 a	83.75 a	80.00 a	83.75 a	73.75 ab	85.00 a	90.00 a	80.00 a
40 °C	20.00 bc A–C	20.00 b A–C	18.75 bc BC	16.25 c BC	36.25 bc AB	47.50 b A	1.25 b C	10.00 c BC

Within each column, values with different lower cases, when present, indicate significant difference at $p < 0.05$ (Tukey's test). Within each row, values with different upper cases, when present, indicate significant difference at $p < 0.05$ (Tukey's test).

3.4. Mean Germination Time under Controlled Temperatures

The germination speed was significantly affected by temperature ($p < 0.001$), and at 32 °C, the seeds germinated faster (MGT 3.14 d) than those at the optimal temperature (4.65 d at 25 °C), similar to the extreme temperature of 40 °C (MGT 4.76). A longer time was needed to germinate at 16 °C (12.05 d). Nevertheless, the longest time was needed at the suboptimal temperature (12 °C), which needed 23.33 days to reach germination (Table 4).

The germination time also significantly varied with the genotype ($p < 0.001$). Seeds of the ‘Local’ castor were significantly the fastest in germination (5.99 d) contrary to the dwarf hybrids that on average had an MGT of 10.10 d. The most delayed in germination was ‘C1013′, which, across temperatures, took more than 11.78 days to complete germination.

The significant T × G interaction ($p < 0.001$) on MGT indicated a different genotype response to temperature for this trait. At optimal (25 °C) or near-optimal (32 °C) temperatures, the dwarf genotypes took much more time to germinate (MGT up to 7.34 in ‘C1013′ and 4.75 d in ‘C857′, at 25 °C and 32 °C, respectively) (Table 6). This pattern was more evident at the optimal temperature, whereas, at 16 °C, ‘C1020′ was the longest with 16.93 d.

Table 6. Mean germination time (MGT, days) in eight genotypes of castor at five temperatures (12, 16, 25, 32, 40 °C) ($n = 4$).

Temperature	Genotype							
(T)	C857	C1008	C1012	C1013	C1018	C1019	C1020	Local
12 °C	23.00 a B	30.67 a A	16.00 a C	28.00 a AB	28.00 a AB	23.00 a B	28.00 a AB	10.00 a C
16 °C	7.97 b C	11.79 b A–C	13.37 a A–C	15.03 b AB	9.93 b BC	11.57 b A–C	16.93 b A	9.90 a BC
25 °C	3.97 c CD	5.46 c BC	3.30 b D	7.34 c A	5.60 c B	5.43 c BC	3.37 c D	2.77 b D
32 °C	4.75 c A	3.53 c AB	3.10 b AB	3.20 d AB	3.60 c AB	2.47 c B	2.70 c B	1.77 b B
40 °C	4.75 c	3.83 c	5.50 b	5.33 cd	4.60 c	3.53 c	5.00 c	5.53 b

Within each column, values with different lower cases, when present, indicate significant difference at $p < 0.05$ (Tukey’s test). Within each row, values with different upper cases, when present, indicate significant difference at $p < 0.05$ (Tukey’s test).

3.5. Synchrony of Germination

The synchrony of germination (Z) indicates the level of synchronization of seed germination. Synchrony, which was quite low in all genotypes, was significantly higher at 25 °C (Z = 0.32, against 0.25 at 32 °C and 0.12 at 16 °C). It also changed with the genotype (p <0.001), and the greatest Z (0.47) was calculated in genotype ‘C1020′. This value was statistically different from the rest of the dwarf genotypes, while the ‘Local’ one was not statistically different from the highest or the rest of the dwarf genotypes. In turn, the lowest Z (0.14) was calculated in ‘C1018′, whose Z did not differ from the rest (Table 4). At the optimal temperature (25 °C), the highest value of 0.63 of synchrony was obtained in ‘C1020′; contrarily, a low value of 0.03 was reached at 16 °C for ‘C1012′, ‘C1013′, and ‘C1018′. At 32 °C, the highest (0.30) was achieved by the ‘Local’ genotype (Table 7).

Table 7. Synchrony of germination (Z) in eight genotypes of castor at three temperatures (16, 25, 32 °C) ($n = 4$).

Temperature	Genotype							
(T)	C857	C1008	C1012	C1013	C1018	C1019	C1020	Local
16 °C	0.07 b B	0.07 B	0.03 b B	0.03 a B	0.03 b B	0.10 a AB	0.53 a A	0.17 c AB
25 °C	0.33 a AB	0.13 B	0.50 a AB	0.27 a AB	0.13 ab AB	0.20 a AB	0.63 a A	0.40 a AB
32 °C	0.20 ab	0.27	0.17 ab	0.27 a	0.27 a	0.27 a	0.27 a	0.30 b

Within each column, values with different lower cases, when present, indicate significant difference at $p < 0.05$ (Tukey’s test). Within each row, values with different upper cases, when present, indicate significant difference at $p < 0.05$ (Tukey’s test).

3.6. Base Temperature and Thermal Time

A linear model was used to estimate the critical germination temperature, based on the germination rate GR_{50} (i.e., $1/t_{50}$). As above mentioned, data at 12 and 40 °C were excluded from the calculation because of the low level of germination, as well as 'C1012′ and 'C1020′, because a germination lower than 50% occurred at 16 °C. The average minimum or base temperature allowing germination of the genotypes was 12.5 °C (Table 8). The base temperature varied with the genotype, from 12.1 °C ('Local') to 12.8 °C ('C1008′). It is interesting to notice that all genotypes exhibited a similar Tb (>12 °C), with just a slight variation among them. Nonetheless, they presented different germination rates (i.e., b slope of linear regression of GR_{50} vs. T), revealing a faster or slower response and sensitivity to increasing (or decreasing) temperatures. In depth, the 'Local' genotype, being the most tolerant to the lowering of temperature (83.75% germination at 16 °C), had the lowest Tb 12.1 °C. The genotype with the highest Tb value was 'C1008′, having 90% of germination at 25 °C, with a 30.55% decrease (62.50%) at 16 °C. In this regard, it resulted as the most sensitive to the lowering of temperature.

Table 8. Values of base temperature (*T*b), estimated (from the model ± σ) and calculated (inverse of the slope b) thermal time θ_T in six genotypes of castor. Data of 'C1012′ and 'C1020′ were excluded because levels of germination (<50%) were reached at 16 °C.

Genotype	***T*b (°C)**	**θ_T (°Cd) (From Model)**	**θ_T (°Cd) (1/b)**
Local	12.1	33.9 ± 2.49	32.8
C857	12.6	46.3 ± 1.98	46.7
C1008	12.8	58.8 ± 6.44	59.5
C1012	-	-	-
C1013	12.7	77.3 ± 2.88	76.3
C1018	12.3	52.4 ± 5.47	57.4
C1019	12.4	46.6 ± 6.95	47.3
C1020	-	-	-
Average (±σ)	12.5 ± 1.0	52.6 ± 4.3	53.4 ± 4.3

Thermal time to 50% germination, as estimated from the model, was on average 52.6 °Cd. It was different among the genotypes, ranging between 33.9 ('Local' genotype) and 77.3 °Cd ('C1013′). This last genotype, although having a similar *T*b to that of the other genotypes, had high thermal time requirements, revealing a slow germination speed. Values of θ_T as estimated from the model closely matched those calculated (from the inverse of the slope b of x-axis intercept of Gr_{50} vs. T). When calculated as 1/b, the average θ_T was 53.4 °Cd, close to the model value.

3.7. Radicle Length

The mean effects of the two experimental factors (temperature and genotype) on the radicle length of castor are summarized in Table 4. At the optimal temperature (25 °C), seeds developed a radicle that, by the fourth day, across genotypes, was 1.96 cm long. Interestingly, at a higher temperature (32 °C), seed radicles reached significantly longer lengths (T, $p < 0.001$) than those measured at 25 °C (4.54 cm). However, a further thermal increase to 40 °C slowed the radicle growth, and at this temperature, the mean length was 0.83 cm. Lower temperature negatively affected the radicle length, which reached 0.60 and 1.50 cm at 12 and 16 °C, respectively.

'Genotype' also significantly influenced the radicle length (G, $p < 0.001$). Across temperature, the fastest radicle elongation was measured in 'C1020′ and the 'Local' castor, along with 'C1012′, whose seeds developed radicles that, by the fourth day from the appearance of the first radicle, were ≥2.36 cm. Much smaller radicles (≤1.88 cm) were developed by the remaining genotypes. Among the remaining genotypes, the lowest value (1.13 cm) corresponded to 'C1013′.

The significant T × G ($p < 0.001$) on the length of radicles revealed that the radicle growth changed among the different genotypes with temperature. At the optimal temperature (25 °C), the longest radicles were measured in 'C1012′ and 'C1020′ (3.07 and 2.67 cm, respectively) and the smallest developed in 'C1013′ (0.67 cm) (Table 9). The increase in temperature to 32 °C boosted the radicle growth in all genotypes, with more remarkable effects in 'C1008′, whose seeds developed a radicle that was threefold longer (>4.60 cm) than that measured at 25 °C. In this genotype, both a thermal decrease and increase to 12 and 16 °C and 40 °C, respectively, strongly impacted the seedling growth, and the final radicle was 0.67 cm at 12 °C, 1.27 cm at 16 °C, and 0.70 cm at 40 °C.

Table 9. Radicle length (cm) in eight genotypes of castor at five temperatures (12, 16, 25, 32, 40 °C) (*n* = 4).

Temperature	**Genotype**							
(T)	**C857**	**C1008**	**C1012**	**C1013**	**C1018**	**C1019**	**C1020**	**Local**
12 °C	1.40 A	0.67 b A–C	0.40 d BC	0.17 d C	0.23 c BC	0.13 d C	0.33 d BC	1.53 bc AB
16 °C	1.80	1.27 b	2.13 bc	0.63 c	0.97 b	1.77 bc	1.87 bc	1.60 bc
25 °C	2.43 AB	1.63 b AB	3.07 b A	0.67 bc B	1.47 b AB	1.97 b AB	2.67 b A	1.83 b AB
32 °C	2.43 D	4.60 a C	5.20 a BC	3.20 a D	2.60 a D	5.10 a BC	7.30 a A	5.90 a B
40 °C	0.80 AB	0.70 b AB	1.00 cd A	1.00 b A	0.87 bc AB	0.43 cd B	0.90 cd AB	1.00 c A

Within each column, values with different lower cases, when present, indicate significant difference at $p < 0.05$ (Tukey's test). Within each row, values with different upper cases, when present, indicate significant difference at $p < 0.05$ (Tukey's test).

3.8. Vigour Index (VI)

As mentioned in Table 1, the vigour index (VI) is calculated by multiplying the germination percentage (%) and the radicle length (cm). The mean values obtained are shown in Table 4. Statistically, VI showed a significant difference in relation to temperature, genotype, and their interaction ($p < 0.001$).

At suboptimal (12 °C) and supraoptimal (40 °C) temperatures, VI had the lowest values (10.98 and 16.47, respectively), whereas 16 °C had higher values in comparison with these last two temperatures but lower than at 25 and 32 °C, reaching a VI of (90.85). Interestingly, VI was maximized at 32 °C, with a value (381.21) that was significantly greater than that calculated at the optimal temperature (25 °C, VI = 177.39). VI also varied with genotype, being the highest in 'C1020′ (207.10) and 'Local' (167.07) and the lowest in 'C1013′ (71.30) and 'C1018′ (81.73). The significant T × G interaction revealed different VIs calculated for the genotypes of castor, depending on temperature. Specifically, at 25 °C, wide variability was noticed within the dwarf genotypes, whose VIs significantly ranged between 43.70 ('C1013′) and 267.57 ('C1012′) (Table 10). This did not occur at 32 °C, where the dwarf genotypes widely differed in VI (from 192.50 in 'C1018′ to 654.75 in 'C1020′). At this temperature, the highest VI was found for 'C1020′. Overall, the greater VI at 25 °C in some genotypes did not correspond to a greater VI at 32 °C for the same genotypes. The 'Local' castor showed the same trend as the dwarf genotypes, having a greater VI at 32 °C (473.60), decreasing to a minimum of 9.87 at 40 °C. Contrarily, having a comparison with the dwarf genotypes, the 'Local' genotype had higher values for the lowest temperature (46.10 at 12 °C and 132.20 at 16 °C), showing greater tolerance to low temperatures.

Significant differences were highlighted among the genotypes studied at 32 °C for the VI. In particular, the highest values occurred for 'C1020′ (VI, 654.75) and 'Local' (VI, 473.60), followed by 'C1019′ (439.40), while the lowest value was obtained for 'C1018′, with 192.50.

Table 10. Vigor index (VI) in eight genotypes of castor at five temperatures (12, 16, 25, 32, 40 °C) (*n* = 4).

Temperature	**Genotype**							
(T)	**C857**	**C1008**	**C1012**	**C1013**	**C1018**	**C1019**	**C1020**	**Local**
12 °C	18.07 b B	6.90 c B	7.93 c B	1.67 d B	2.33 c B	1.47 d B	3.33 c B	46.10 c A
16 °C	68.97 ab A–C	76.99 bc A–C	88.13 c A–C	28.90 bc C	59.73 bc BC	160.18 bc A	111.69 bc A–C	132.20 b AB
25 °C	212.03 a AB	148.78 b AB	267.57 b A	43.70 b B	122.04 b AB	191.66 b AB	259.76 b A	173.60 b AB
32 °C	226.37 a DE	379.42 a B–E	417.20 a B–D	266.41 a C—E	192.50 a E	439.40 a BC	654.75 a A	473.60 a AB
40 °C	19.17 b	14.10 c	15.83 c	15.83 cd	32.07 c	18.93 cd	5.95 c	9.87 c

Within each column, values with different lower cases, when present, indicate significant difference at $p < 0.05$ (Tukey's test). Within each row, values with different upper cases, when present, indicate significant difference at $p < 0.05$ (Tukey's test).

4. Discussion

Slight genetic variability was observed in the 100-seed weight, with an average of 37.01 g. This value is higher than that reported by Wang et al., 2010 [22], who evaluated 1033 accessions of castor seeds with a mean of 28.3 g. This is also similar to what was reported by Santiago et al., 2023 [23], who, by evaluating sixteen Mexican varieties of castor, found a higher seed weight (49.31 g per seed), instead of 31.45 g in some commercial varieties. Negligible differences were highlighted concerning the genotype, indicating a minor influence on this trait.

In this study, the course of germination with time was effectively described using a two-parameter sigmoidal curve. Zhu et al. 2019 [24] adopted a similar function to describe the seed germination time course in sweet-sorghum seeds under salinity stress. After an initial phase of low germination, in which water and oxygen are absorbed through the seed coat [25], seed germination speeds up, until its maximum (which differs with the genotype and temperature) is reached. The same trend of germination was observed at the optimal (25 °C) and near-optimal temperature (32 °C), and, in some cases, the curves for the two temperatures overlapped. This result indicates that in some genotypes, germination at 25° and 32 °C started and proceeded simultaneously. On this basis, we may state that the optimal range of temperature for germination in castor may be extended over that (25 °C) reported in the literature [11].

On the whole, a temperature of 16 °C ensured optimal levels of germination, even if it started later than at 25 °C. The supraoptimal temperature (40 °C) and suboptimal temperature (12 °C) negatively affected germination in all genotypes. Indeed, as confirmed by Severino et al., 2012 [8], inauspicious climatic conditions (i.e., low soil temperature) are considered the most influential factors for the slowing down and inhibition of castor seeds' germination.

As confirmed by Marcos-Filho, 2015 [26], the importance given to seed germination is related to the establishment of crops and consequently to the yield obtainable. Overall, seed germination at 25 °C was slightly lower in the dwarf genotypes (mean, 87.32%, against 92.50% in the 'Local'). This is similar to what was reported at 16 °C (60.85 and 83.75% for the dwarfs and 'Local' genotype, respectively). However, this did not occur at 32 °C, where the dwarf genotypes had slightly higher values than the 'Local' castor (83.21 against 80.00%, respectively). These results seem to indicate the strong influence of the genotype factor on the FGP, matching what was reported by Amorim Neto et al., 2001 [27] who accomplished germination ranging from 84.00 to 86.70% in dwarf castor. Moreover, considering that seed germination on average achieved a level $\geq$ 82.81% at 32 °C, the optimum for germination in castor may be extended to this temperature, as validated by Severino et al., 2012 [8] who reported that a temperature of 31 °C is the optimum for germination in castor. In addition, in this context, the dwarf genotypes, selected for arid and semiarid environments, are more adapted and tolerant to higher temperatures, as also confirmed at 40 °C, in which the dwarf genotypes, being more acquainted with higher temperatures, achieved 22.86% of germination against 10.00% of the 'Local'. Nonetheless, Falasca et al., 2012 [28] reported that temperatures > 30 °C represent a limit for castor cultivation, because seeds of castor may fail to germinate.

The 'Local' genotype, which achieved similar results at both 25 and 32 °C (FGP > 80%), was also the only genotype that reached 30.00% germination at 12 °C and reached 83.75% at 16 °C, indicating a greater tolerance of this genotype to suboptimal and lower temperatures, attributable to the Tunisian environment from which it was selected, where low night temperatures characterise the desert area.

In this experiment, no germination occurred at 8, and at 12 °C, most of the genotypes failed to germinate (level lower than 5% of germination). These results indicate that the thermal requirements for castor germination, at least for the dwarf hybrids examined, are above the minimum (15 °C) reported by Severino et al., 2012 [8], and that the base temperature is below that suggested (15–16 °C) by Amorim Neto et al., 2001 [27] and

Moosavi et al., 2022 [29], who found that castor may germinate even at temperatures ranging between 5 and 15 °C.

In this regard, seeds present a critical temperature for germination, meaning that seeds, which have a base temperature (*T*b) above T, may fail in germination [21]. The necessity of studying the base temperature is related to the acknowledgment of the thermal stress that causes the seeds to reach out, affecting their establishment. The lower the *T*b, the greater the tolerance to stress given by a lowering of temperature. Our findings revealed that castor seeds had a different behaviour when exposed to low temperatures, in terms of germination rate and speed. Indeed, the 'Local' castor resulted as the best genotype for early sowings, having the lower *T*b, indicating a greater tolerance to thermal stress. This result matched those of the FGP, with the 'Local' genotype, the only one among the dwarf hybrids, achieving 30.00% germination at 12 °C.

Several studies confirmed that the seed radicle requires a lapse of time to break the testa, and the length of this period depends on the soil temperature [1]. Koutroubas et al., 1999 [30], working on castor grown in two different sites, noticed that in the warmer location, seeds took 2 to 19 days less than in the cooler site to germinate. In the present study, at 25 °C, seeds germinated in 2.77 ('Local') to 7.34 ('C1013′) days, and at 32 °C, germination occurred even 1.51 days earlier than at 25 °C.

Kittock et al., 1967 [31] and Weiss, 2000 [32] reported a germination time of 25 days and 10–23 days, respectively, for the germination of castor in a range of soil temperatures between 10 and 19 °C as confirmed by our results, in which, at 16 °C, seeds took from 7 to 15 days to germinate. In our study, seeds took 10 to 30 days to germinate at 12 °C, but at this temperature, germination was low in most cases. This could be explained by the fact that germination falls sharply when temperatures are reduced from the optimal, because low temperatures slow down metabolic and enzyme activities, affecting the whole germination process [33]. According to Patanè et al., 2009 [34] low temperatures prolong the initial phase (lag phase) of germination, extending the germination time. Seeds of 'Local' castor germinated slightly faster than the dwarf genotypes, indicating that the germination speed may depend on the genotype.

The indication of synchrony is useful for predicting the degree of success of a crop in terms of plant establishment [18]. Z = 1, which is obtained when all the seeds germinate on the same day, indicates simultaneous germination, while values closer to 0 indicate a low level of synchronization. In this research, the highest temperature (32 °C) showed a lower level of synchrony compared to 25 °C. It seems that high temperatures affect the synchrony of germination in castor. Patanè et al. 2021 [21] obtained a better result at 25 °C, confirming our results. The values obtained by Windauer et al. 2012 [33] in *Jatropha curcas* L. are comparable to our data for synchrony at 25 °C.

Another parameter considered is radicle growth. As reported by Wolny et al., 2018 [35], the beginning of radicle growth starts when germination finishes. Temperature influences the radicle growth process, and in this study, any thermal increase or decrease from the optimum temperature (25 °C) had evident effects on this trait. Indeed, an increase in temperature from 25 to 32 °C accelerated the growth of radicles that, 4 days after the first visible radicle appeared, were 4.54 cm long, on average, among all genotypes, against 1.96 cm measured at 25 °C. According to Patanè et al., 2009 [34] really low and low radicle growth that occurs at 12, 16, and 40 °C may be ascribed to alterations in the metabolic processes that are strictly temperature-dependent. The same restrictive effects on radicle growth were reported in seeds of *Brassica napus* L. [36].

Through the analysis of the vigour index, it is possible to evaluate the differential expressions in the crops' response to different external factors (i.e., temperature) [37]. The vigour index resulted highest at 32 °C followed by the VI at 25 °C; lower values were obtained at 12, 16, and 40 °C. Sharma et al., 2022 [38], in wheat, noted differences in the VI, determined by temperature, reporting a greater VI at 25 °C than at 32 °C. This could be explained by the fact that wheat is a micro-thermal crop, contrary to castor, which is a warm season crop and thus has a higher vigour index when temperatures increase.

5. Conclusions

As a warm season crop, castor was found to germinate well at temperatures ranging from 25 to 32 °C, where seed germination exceeded 82.00% at both temperatures. Decreases and increases in these temperatures negatively affected the seed germination, which was reduced by 90.05% at 12 °C, by 27.56% at 16 °C, and by 75.84% at 40 °C, indicating that seeds of castor may effectively germinate within a rather restricted range of temperatures.

The impact of the genotype strongly influenced all the germination parameters. Indeed, the 'Local' castor, selected for good adaptation to the Mediterranean environment, had greater tolerance to low temperature than the dwarf genotypes, which prefer higher temperatures and are better adapted to late sowings. With the 'Local' castor being the most tolerant to the lowering of temperature, it is suggested for early sowings in spring.

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