Optimal Germination Temperature Assessed by Indices and Models in Field Bean (*Vicia faba* L. var. *minor*)

Khalil KHAMASSI [1] (✉)
Kalthoum HARBAOUI [1, 3]
Jaime A. TEIXEIRA DA SILVA [2] (✉)
Fayçal Ben JEDDI [1]

Summary

This paper seeks to shed light on the effect of temperature on field bean (*Vicia faba* L. var. *minor*) germination and root elongation. Accordingly, the results were as follows: germination was faster at 20°C than at 5, 10, 15, 25 or 30°C. However, temperature had no effect on total germination percentage between 10 and 30°C, although it was reduced by approximately 4% at 5°C and delayed at other temperatures. 50% germination required nine days at 5°C but only three days at 20°C. Seeds required a mean germination time of 12 days for total germination at 5°C but only four days at 20°C. The coefficient of the velocity or rate of germination index reached the highest (23.71%) and lowest (8.24%) value at 20°C and 5°C, respectively. Maximum germination speed (43.26), as assessed by the speed of germination index, was at 20°C. The speed of accumulated germination grew in proportion to the increase in incubation temperature. The main root axis length index and root elongation speed peaked at 20°C (five days and 0.72 cm/day, respectively). Prediction of optimal temperature for germination (To) by using the equation $D = [a-(b\times T) +(c\times T^2)]$ showed that 20°C is the optimum To, indicating that the theoretical model was robust. Hence, it could confidently be used to calculate all parameters related to the effect of temperature on faba bean germination.

Key words

field bean, *Vicia faba* L. var *minor*, germination, temperature, root elongation, modeling

[1] Laboratoire des Sciences Horticoles, Institut National Agronomique de Tunisie, 43 Av. Charles Nicolle 1082 Tunis- Mahragène, Université de Carthage, Tunisia
✉ e-mail: khkobe@hotmail.com
[2] P. O. Box 7, Miki-cho post office, Ikenobe 3011-2, Kagawa-ken, 761-0799, Japan
✉ e-mail: jaimetex@yahoo.com
[3] Regional Field Crops Research Center, B. P. 350 Béja 9000 - Tunisia

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Introduction

Since the term germination is often imprecisely – and in some cases wrongly – used, it can lead to confusion regarding what is considered to be seedling growth, which typically starts at the end of germination (Bewley and Black, 1994; Ranal and Santana, 2006). Indeed, Bewley and Black (1994) defined germination as a process that starts with water hydration by quiescent dry seeds and ends with the appearance of a radical or embryonic axis. Several previous studies have dealt with the importance of studying some quantitative features which may be related to controlling seed activity during this process (Labouriau, 1983a; Brown and Mayer, 1988; Bewley and Black, 1994). Ranal and Santana (2006) affirmed that time, rate, homogeneity, and synchrony are important, particularly in an ecological context, to estimate the degree of success of a species, since the spread of harvested seed and its subsequent germination would allow for the special and temporal selection of plantlets suitable to multiple environments. In addition, in an agricultural context, farmers prefer uniform germination and sprout emergence (Balkaya, 2004). Yet, temperature also has an effect on the different stages of germination. Kurtar et al. (2004) noted that germination was more affected by temperature than by other factors while Nerson (2007) claimed it to be the most significant factor. This effect can be expressed as cardinal temperatures [minimum, optimal and maximum temperatures for germination] (Copeland and McDonald, 1995). Studies on broad bean (*Vicia faba* L. var. *major*) have shown that the minimum, optimum and maximum temperatures were 3, 20-25 and 30-35°C, respectively (Sehirali, 1988; Sepetoglu, 1994). Temperature limits for germination might define some ecological restrictions for the geographic spreading of a species (Kurtar, 2010). Kay (1979) claimed that the optimum growth temperature for so-called Mediterranean faba bean (*Vicia faba* L.) varies between 18 and 27°C. In fact, these types are well adapted to winter planting in subtropical conditions, including low altitudes in the Mediterranean region (Loss and Siddique, 1997; Loss et al., 1997a; Sau and Mínguez, 2000; Santonoceto et al., 2001). There is a significant difference in growth between faba bean cultivars in terms of the optimum temperature needed for seed sprouting with the optimum ranging from 24 to 30°C (Norouzi and Vazin, 2011). Manschadi et al. (1998) suggested that the optimum temperature for faba bean sprouting occurs between 24-28°C and 27°C. Ellis et al. (1987) also reported that the desirable temperature for sprouting in different faba bean cultivars was, as assessed by linear regression, between 20 and 26°C. To assess the effect on faba bean germination, Said et al. (1967) adjusted night temperature between 5 and 20°C and used constant day temperature of 20°C in a growth chamber, discovering that the optimum value changes within the growth stage up to the 2-4 leaf stage and that stem elongation was fastest at 20°C, which was coincidentally also the highest night temperature. In addition, germination studies conducted in the laboratory are useful to understand field emergence. Accordingly, field emergence data from ICARDA's farm for a large number of Mediterranean types in north Syria showed that temperatures below 10°C resulted in a conspicuous delay in days to emergence. Sutcliffe (1977) asserted that temperature affected both mitosis and cytokinesis in meristematic tissues. As such, germination proceeded very slowly at temperatures below 3°C and most rapidly at 25°C in faba bean root meristems. This would henceforth be useful for understanding the effect of low temperature on root elongation. In all these studies, however, statistical analyses or correlation studies were not performed. This study thus sets out to define the optimum temperature for germination of field beans (*Vicia faba* var. *minor*) by means of different indices in order to single out the most efficient index to study field bean seed germination. Moreover, temperature was assumed to be the main factor affecting germination and seedlings since soil moisture in winter sowing is not a constraint in Tunisia. Furthermore, field bean is sown in Tunisia between mid-September and early January. The soil temperature from September to December is around 28°C and then decreases to ~ 7°C by December. This affects the homogeneity of field emergence and, later on, yield. This paper also seeks to evaluate seed quality through the germination of a Tunisian field bean landrace in order to predict its possible success in different ecological regions by experimentally predicting the effect of temperature on field emergence. The tested material, which was collected during the 1970s and which was considered as a unique cultivated population at that time, has been maintained and purified in the National Institute of Agronomy of Tunis (INAT) mini gene bank. This material is currently used as important germplasm in both Tunisian and British pre-breeding projects..

Materials and methods

The seeds used in this study consisted of pure lines developed by a process of purification by a single plant progeny of an old Tunisian landrace 'F75' of field bean (*Vicia faba* L. var. *minor*) conserved in the Agronomy laboratory at INAT. Optimum germination temperature was 20°C according to ISTA (1999).

Germination test

Germination was performed in a vacuum oven (VO, Memmert, WI, USA) in which 50 seeds were positioned on or placed between two layers of soaked Whatman filter paper No. 1 (ISTA 1999) in 15-cm-diameter glass Petri dishes. The same trial was carried out, in the dark, at six different temperatures (5, 10, 15, 20, 25, and 30°C). There were three replicates of each treatment in a completely randomized design. Distilled water was regularly added to each Petri dish to replace any evaporated water. The seeds were sowed daily and were considered to be germinated when the emerged radicle elongated to 1 cm, according to Samimy et al. (1987). The main root axis length (MRAL) was measured in cm with a ruler.

Germination indices

Six germination indices were calculated (Table 1): Germination capacity (GC), 50% germination (T_{50}), Mean germination time (M-days), Coefficient of the velocity or rate of germination (CRG), Speed of germination (S) and the Accumulated germination speed (AS). To better understand AS, we calculated the average germinated seed per day (AGSD). Data were statistically analyzed using Excel 2010 software to calculate indices and modeling.

Model development procedure

A model $D = [a-(b\times T) + (c\times T^2)]$, developed by Uzun et al. (2001), was used to calculate the optimal temperature for germination (To) for field bean by a solver procedure as an added Excel package computer program. In the model, D = the time passing from seed sowing to emergence based on temperature

Table 1. Germination indices tested in this study

Germination index	Formula	Definition	References
GC Germination capacity	Gt = [NT × 100]/N	NT: proportion of total germinated seeds in each treatment N: Number of seeds used in the bioassay	Commonly used
T_{50}	Days required for 50% germination of the total number of seeds		Orchard (1977); Josep and Maria (2002)
AGSD Average number of seeds germinated per day	ASGD = NT/T	N: Total number of germinated seeds T: total number of days required for final germination	The authors' own calculation
M-days mean germination time (in days)	M-days = [(N1 × T1)+...+ (Nn × Tn)]/ [N1 + N2 + N3 +... + Nn]	Tn: proportion of total germinated seeds in each treatment N: Number of seeds used in the bioassay	Edmond and Drapala (1958); Czabator (1962); Ranal and Santana (2006)
S Speed of germination	S = (N1 × 1) + (N2 – N1) × 1/2 + (N3 – N2) × 1/3 + … (Nn – Nn-1) × 1/n	N1, N2, N3, Nn-1, Nn: Proportion of germinated seeds observed at first, second, third … (n – 1), (n) days or hours	Bradbeer (1988); Wardle *et al.* (1991)
AS Speed of accumulated germination	AS = [N1/1 + N2/2 + N3/3 … + Nn/n]	N1, N2, N3, Nn: Cumulative number of seeds which germinate at time 1, 2, 3, ... N	Bradbeer (1988); Wardle et al. (1991); Haugland and Brandsaeter (1996)
CRG Coefficient of the of velocity or rate of germination	CRG = 100 × ([N1 + N2 + N3 +... + Nn]/[(N1 × T1) +...+ (Nn × Tn)])	N1: number of germinated seeds at time T1 N2: number of germinated seeds at time T2 Nn: number of germinated seeds at time Tn	Bewley and Black (1985)

Table 2. Germination indices at different incubation temperatures

Temperature (°C)	T_G	M-days	T_{50}	S	AS	ASGD	CRG (%)
5	145	12.13	9	15.38	77.94	10	8.24
10	150	6.59	5	28.39	83.23	18.75	15.16
15	150	5.67	4	36	78.9	21.42	17.62
20	150	4.21	3	43.26	89.75	30	23.71
25	150	4.69	4	24.68	97.61	25	21.29
30	150	6.09	4	17.74	107.64	18.75	16.4

Legend: Germination capacity (GC), 50% germination (T_{50}), mean germination time (M-days), coefficient of the velocity or rate of germination (CRG), speed of germination (S), accumulated germination speed (AS). To better understand AS, we calculated the average germinated seed per day (AGSD).

variation (days), as pointed out precisely by Uzun et al. (2001), T = mean temperature (°C) and a, b and c = the coefficients. The proportion of variation in seed germination was obtained from a derivative of the above equation: Dd/dt = -b + (2×c×T). If the degree of variation is zero, then another equation determining To can be obtained. At that point, the equation tends towards To = b/2×c. The equation predicting To obtained in this study was compared with equations reported in previous studies.

Effect of temperature on main root length

Measured by a ruler in cm, MRAL represented the average root length of 15 germination days for three replicates per temperature treatment from 5 to 30°C.

Results and Discussion

Germination capacity

There was no effect of incubation temperature on the total number of germinated seeds except for 5°C at which 96% of tested seeds (145/150 seeds) germinated (Table 2). Incubation temperature did not affect GC but it had an effect on the number of days to reach 100% germination. However, at 5°C, only 96% of seeds germinated, which can be explained by the quality of the testa. Typically, injury to grain legume testae is associated with imbibition of water at low temperatures, inducing transverse cracking of the cotyledons (Dickson et al., 1973; Hobbs and Obendorf, 1972).

Germination time estimates

The T_{50} or number of days to reach 50% germination (Table 2) required nine days at 5°C but three days at 20°C (Fig. 1). Seeds required 12 M-days for total germination at 5°C while the best results were at 20°C with four M-days. Two indices (T_{50} and M-days) showed that To is at 20°C. These results are in agreement with those of previous studies. Indeed, M-days, according

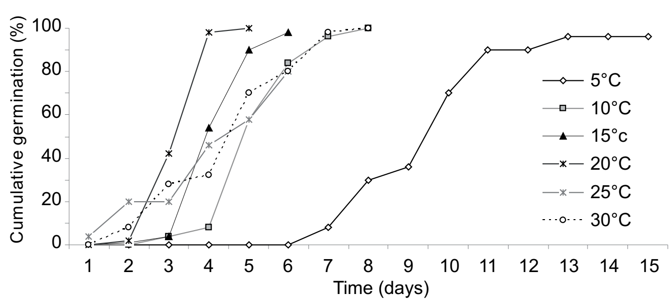

Figure 1. Cumulative germination at different incubation temperatures based on the average of 150 seeds per treatment

to Czabator (1962), is the average length of time necessary for the maximum germination of a seed lot and it can have the same units of time used for calculating germination (hour or days). T_{50} is defined as the median thermal time or median germination time (Bayuelo-Jimenez et al., 2002). Based on Czabator's work (1962), Costa Neto (1977) and Ranal and Santana (2006) pointed out that when germination distribution is symmetric, M-days and T_{50} both represent the central tendency of the data. Otherwise, T_{50} would be more appropriate than M-days.

Germination rate

The germination rate will be discussed in terms of CRG or the coefficient of velocity, S and AS (Table 2). The highest CRG index was noted at 20°C while the lowest was at 5°C. The highest CRG value was registered at 20°C in this study. This index explains precisely the velocity and spread of germination during the whole period of germination. Furthermore, CRG is estimated by the same formula suggested by Maguire (1962) and which is applicable in both field and laboratory experiments. The high value of this index provides information about the vigor of seed samples (Ranal and Santana, 2006). The S index indicates that the maximum speed of germination was at 20°C. Furthermore, this index indicated that temperatures below 10°C and above 25°C decreased the speed of germination but did not halt it. AS increased in proportion to the increase in incubation temperature. This index gives an idea about the progress of cumulative germination during the incubation period (15 days), except at 15°C. On the other hand, it also represents the cumulative speed of germination per day. Indeed, the highest number of germinated seeds in the incubation period (15 days) was between 10 and 25°C with an optimum level at 20°C. Moreover, few seeds germinated each day when incubation temperature was less than 10°C. The highest AS value was obtained at 30°C although another index, namely AGSD, was used and which indicated that 20°C resulted in the fastest speed of germination. The difference between AGSD and AS is considered: the former represents the speed of germination taking into consideration the total incubation period and the total number of germinated seeds, while the latter represents the cumulative germination speed per day in which new germinated seeds are observed.

Estimate of To for T_G and confirmation of the model equation

To determine the adapted seed germination model for faba bean variables (Table 3), a simulation led to the model $D = [a-(b\times T)+(c\times T^2)]$. The predicted optimal temperature in this model is 19.2°C, which provided the fewest number of days to achieve germination (Table 3 and Fig. 2). The predicted To was very close to the observed optimum (To), which confirms the accuracy of the model used. Minimum, optimum and maximum germination temperature in this study were 5, 20 and 30°C, respectively that were used to predict To, confirming the model as suggested by Uzun et al. (2001). As such, it allowed the prediction of To from independent variables (a, b and c) resulting from the equation (Table 3). The predicted To agrees with the Balkaya (2004) model value.

Figure 2 illustrates D as the time passing from seed sowing to emergence based on temperature variation (days) and the predicted D for each temperature treatment. The curves are almost identical except for a minor difference registered at 25°C that corresponds to a higher value of D than the predicted D value by approximately four days. Most models based on laboratory experiments under controlled conditions and modeling based on field conditions are still not well documented (Hardegree et al., 2003; Kurtar, 2010).

Table 3. The coefficients a, b and c for model $D = [a-(b\times T)+(c\times T^2)]$ and the optimal predicted To

Coefficient predicted			Optimum temperature To (°C)	
A	B	C	Predicted by the model	Indicated in the literature (analysis)
15.16649	-0.97972	0.023094	19.20238	18.2 (Balkaya, 2004)

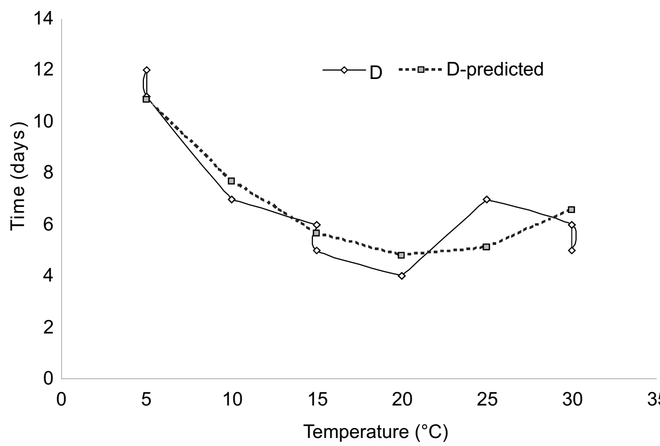

Figure 2. Relationship between temperature and cumulative germination during the period based on the average of 150 seeds per treatment. D = time (days).

The effect of temperature on main root length

MARL was no longer measured when seeds achieved 100% germination in all three replicates per treatment. The average MRAL (Table 4) shows that at 20°C root length reached 4 cm after only five days' incubation with the highest speed at 0.72 cm/day (Fig. 3) and with the lowest coefficient of variation (CV). A high MRAL value was registered at 5°C since measurements were extended to 15 days. The lowest speed of root elongation was at 5 and 10°C with a speed of around 0.1 cm/day, but root elongation did not cease. However, at 30°C, speed decreased to about 0.2 cm/day. The low temperature (5°C) is not suitable for field bean as it takes 15 days to reach the same root length obtained in only five days at 20°C. This result can be useful when considering the environmental conditions in the Mediterranean region in terms of choice of sowing date to allow the plant optimal conditions for rapid emergence and a high rate of germination. Low temperatures (≤5°C) appear to halt vegetative growth (Ellis and al., 1987; Nourouzi and Vazin, 2011) while tempera-

Table 4. Root length at different temperatures

Temperature (°C)	5	10	15	20 (control)	25	30
Number of days of incubation	15	8	6	5	8	6
Mean total root length MRAL (cm)	3.1	0.9	2.1	3.6	4.4	1.4
Standard deviation	0.45	0.15	0.31	0.06	0.29	0.15
CV %	14	16	15	2	6	11

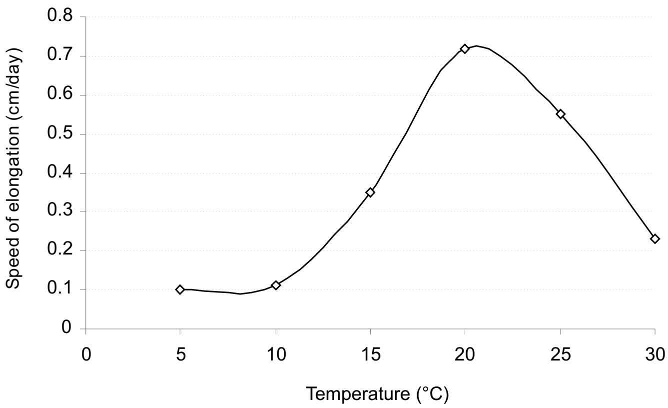

Figure 3. Root elongation speed (cm/day) at different temperatures based on an average of 150 seeds per treatment

tures greater than 25°C tend to decrease root elongation speed. Temperatures between 10 and 25°C affect the ability of a seedling to develop a root system rapidly after germination that is critical for the successful establishment of a plant, as reported by Mosjidis (1995) for *Vicia* spp.

Conclusion

The results of this study showed that 20°C is the optimum temperature for germination and root growth of field bean (*Vicia faba* L. var. *minor*). All indices except for AS were useful to give a precise description of the incubation temperature effects on the germination process. A comparison of different indices may provide better justification regarding seed quality and might give early indications of the success of field emergence, while varying the temperature above or below the optimum (20°C) under laboratory conditions might provide a better understanding of its success under field conditions. Several of these models are used by scientists to predict vegetative growth and yield and some of them have accurately described germination, speed, duration and field emergence in some legumes (Uzun and Ozkaraman, 2000). The current adapted model allowed for the prediction of optimal temperature and could be used to adjust sowing time according to regions and season. Since field bean is currently sown in autumn in Tunisia, spring sowing is suggested based on the results of our findings.

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