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# ANALYSIS OF GERMINATION AND ESTIMATION OF CARDINAL TEMPERATURES IN FABA BEANS

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**SUMMARY** : *The germination at constant temperatures of faba bean cv. Albatross was investigated between 3 °C and 32.5 °C on a thermal gradient plate. Several mathematical functions, namely Weibull, Logistic and two other equations, were fitted to the germination data. Cardinal temperatures and thermal time requirements were calculated using The Weibull and Weaver's functions from which a better fit to the data was obtained. Both models closely reconstructed the acutual data and estimated cardinal temperatures. But, The Weibull function was superior to Weaver's model in terms of quick convergence.*

## BAKLADA ÇİMLENMENİN ANALİZİ VE KARDİNAL SICAKLIK DEĞERLERİNİN TAHMİNİ

**ÖZET** : *Bakla cv. Albatross'un çimlenmesi 3-32.5 °C arasında Değişken Sıcaklık Tabakasında sabit sıcaklıklarda araştırılmıştır. Çimlenme verileri Weibull, Logistic ve diğer iki matematiksel modele göre analiz edilmiştir. Kardinal sıcaklık dereceleri ve termal zaman gereksinmeleri en iyi sonuçların alınıdğı Weibull ve Weaver modellerine göre hesaplanmıştır. Her iki model de çimlenme verilerini ve kardinal çimlenme gereksinmelerini yakın olarak tahmin etmiştir. Fakat, Weibull modeli Weaver modeline göre daha çabuk sonuç vermiştir.*

## INTRODUCTION

Cumulative germination progress curves at a given temperature are approximately S. shaped (Tipton, 1984), indicating that germination rate varies with time. This rate is affected by genetic and environmental factors, primaliy temperature and moisture (Kotowski, 1926; Labuda, 1987).

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Mathematical descriptions of all the facets of germination is of importance in helping to evaluate seedling vigour under laboratory and field conditions. Identification of genotypic differences in the cardinal temperatures and thermal time requirements and the rates of germination is of crutial importance for the establishment and the adaptation of a given cultivar (Covell et al., 1986; Mohamed et al., 1988 b). Many indices and formulae have been proposed to express the germination process in biologically meaningful values. However, no attempt to embrace all facets of the progress curve have, as yet, been successful. Single value indices which confound one or two facets of gemination are inherently ambiguous (Bronwn and Mayer, 1988 a). An alternative to these is to fit standard functions such as logistic (Schimpf et al., 1977). Richards (Richards, 1959) and Weibull (Brown and Mayer, 1988 b). However, these have no intrinsic biological interpretation (Richards, 1959) and often have the problem of poor fit.

This study cmopares several matematical formula that have been proposed to better charecterise germination data in other plant species with the Weibull function and investigates the possiblity of using these functions in order to calculate thermal time and cardinal temperatures requirements for faba beans.

## MATERIALS AND METHODS

The progress of germination of Vicia faba L. cv. Albatross (NIAB, 1992) was investigated at 12 constant temperatures ranging from 3.0 to 32.5 °C (± 0.6 °C) on a one-way temperature gradient plate (TGP) (Garcia-Huidobro et al., 1982) at Sutton Bonington campus of The University of Nottingham.

Four replicates of twenty benomyl dressed seeds were germinated on moist sterile sand in 4x11 cm rectangular petri dishes placed across the temperature range (Dumur et al., 1990). Germinated seeds with radicle lengths of 10 mm (Mohamed et al., 1988 a) were counted at six hourly intervals.

Several mathematical equations (Table 1) were fitted to the data. The equations were Logistic, Weibull equations (Brown and Mayer, 1988b), two other equations proposed by Weaver et al., (1988) and Hunter et al., (1984). The logistic is a three, the others four parameter models and all require an initial estimation of parameters to fit the cumulative germination curve.

The approach of Hunter et al., (1984) uses the number of germinated seeds and is based on explicit probabilistic assumptions ad aims to summarize the main wave of germination, assuming that early germinating seeds are likely to have a greater planting value. The data were analyzed using GENSTAT package devised by

Brain and Butler (1988).

The criteria for testing the goodness-of fit of the models to the data were their sensitivity to initially estimated parameters, easy convergence (Brown and Mayer, 1988 b), the least residual sum of squares (Tipton, 1984), high coefficient of determination ($R^2$) (Berry et al., 1988) and closely description of the actual data (Dumur et al., 1990).

The time taken to 10, 50 and 90 % total germination ($t_{10,}$ $t_{50}$ and $t_{90}$), the lag phase and spread of germination ($t_{90}$ - $t_{10}$) were calculated for each replicate.

The rates of germination (reciprocal of the time taken for 50 % of total germination to be achieved) from each replicate were regressed against temperature, for those temperatures below and those above the optimal temperature (Mohamed et al., 1988 a; Dumur et al., 1990). The points of intersection on the temperature axis gave the base ($t_b$) on the sub-optimal range and maximum temperature (tm) on the supra-optimal range. The optimum temperature ($t_b$) was given by the points at which the two regression lines intersected (Garcia-Huidobro et al., 1982) Thermal time requirements for germination at sub ($ø_1$) and supra ($ø_2$) optimal temperature in degree-days (° Cd) were calculated from the slope (1/ø) of the linear regression analysis of the rates of germination against different temperatures (Garcia-Huidobro et al., 1982). The relationship was $1/t\ (G)= [T-T_b\ (G)]/\ ø_1\ (G)$ for sub-optimal and $1/t\ (G) = [T_m\ (G) - T]\ Q_e\ (G)$ foor supra-optimal temperatures. 1/ø is equivalent to the slope of the rate-temperature relations. Where t (G) is the time taken for cumulative germination to reach the value G in days, T is temperature (°C), $t_б$ (G) and tm are base and maximum temperatures respectively for the given sub-set (g) of the seed populations. $ø_1$ (g) is thermal time (number of day-degrees above $t_b$ (G) required for the seed fraction G to germinate, where $ø_2$ (g) is a second value of thermal time. Confidence limits (Finney, 1978) were given only for base temperature $t_b$ due to lack of fit of regression lines at supra-optimal temperatures. The total germination data were subjected to the analysis of deviance based on a binomial distrubution with a probit link.

## RESULTS

Of the equations attempted, Logistic was sensitive to initial estimation of parameters and often did not converge (Tablo 2). The equation suggested by Hunter et. al (1984) also had poor convergence and sensitivity to initial parameters.

Re-supplying parameter values brought no improvement in contrast to the easy optimization obtained by Hunter et al., (1984) on their data.

The weibull and Weaver's functions, on the other hand, described the data more closely (Table 2). The Weibull function gave 83 % convergence rates and was insensitive to initial estimation of the parameters.

Table 1. Formula of the Functions Used to Describe Cumulative Germination.

| Function | Formula | Number of free parameters |
|---|---|---|
| Weibull | $Y = A (1 - \exp (- k (t-1)^c)$ | 4 |
| Logistic | $Y = A/ (1+\exp (-kt + 1)$ | 3 |
| Weaver et al. | $Y + A/1 + \exp (-d (\ln (t-1) - b))$ | 4 |
| Hunter et al. | $G (t) = b (\ln (t-1) - m)$ | 3 |

Where Y is the cumulative germination at time t, and A, K, ı, c, m, b, z and d are ampirically derived constants. A is the asymptote, k is the rate of increase, l is the lag in germination and c is a shape parameter. b is a measure of the time to 50 % germination after the end of lag period and d is a measure of the standard deviation of Ln (t-1) and the mean of ln (t-1) in Hunter's method is also related to the slope of G (t) at the 50 % germination. 1/z is the variance of ln (t-1). Values of about 3.6 of c indicates that the germination frequency distribution is essentially symmetric. Greater values suggest negative skewness, whereas lower values indicate a positively skewed distribution.

This compares to a 50 % convergence rate with the Weaver's function which appears relatively sensitive to the initial estimates of parameters. Re-supplying initial parameters for the latter did not improve the optimization. The Weibull function was süperior also in terms of minimizing the residual sum of squares from the non-linear regression analysis and in terms of the coefficient of determination (R2 = 0.98-0.99) across the temperature range compared to Weaver's model (R2 = 0.77-0.99) and in terms of closely describing germination data (Figure 1). Nonetheless, Weaver's function predicted asimptote (A) more closely than did The Weibull function (Figure 2). Therefore, these two equations were chosen for the calculation of cardinal temperature requirements.

Temperature affected the eventual germination (mean deviance ratio is 74.1, P<0.001), most notably at supra optimal extremes (Figure 2). Virtually no germination occvired at 32.5 °C within 816 h.

Table 2. The Fitting of the Functions to a Germination Data Set.

| Weibull/rep. | A | l | k | c | R2 and convergence |
|---|---|---|---|---|---|
| 1 | 0.87 | 52.4 | 0.065 | 1.17 | 0.99 (+) |
| 2 | 1.16 | 43.6 | 0.024 | 2.51 | 0.97 (+) |
| 3 | 1.10 | 19.7 | 0.015 | 4.41 | 0.97 (+) |
| 4 | 1.20 | 47.5 | 0.023 | 2.24 | 0.98 (+) |
| Logistic/rep. | A | l | k | - | R2 and convergence |
| 1 | 0.010 | 67.7 | 0.001 | - | y** (-) |
| 2 | 0.010 | 68.7 | 0.001 | - | y (-) |
| 3 | 0.010 | -1.6 | $9.9x10^{-3}$ | - | y (-) |
| 4 | 0.010 | $3.3x10^5$ | $6.3x10^3$ | - | Y (-) |
| Weaver/rep. | A | l | b | d | R2 and convergence |
| 1 | 99.6 | 53.6 | 3.17 | 2.72 | 0.93 (+) |
| 2 | 102.5 | 34.29 | 3.75 | 4.89 | 0.98 (+) |
| 3 | 128.7 | 15.27 | 4.19 | 5.72 | 0.98 (-) |
| 4 | 98.4 | 37.3 | 4.50 | 3.66 | 0.96 (+) |
| Hunter/rep. | - | l | m | b | Mean deviance and convergence (+/-) |
| 1 | - | 51.4 | 13.63 | 0.175 | 40.89 (-) |
| 2 | - | 48.7 | 1.99 | $0.62x10^{-7}$ | 687.5 (-) |
| 3 | - | 50.7 | 1.70 | 5.70 | 319.4 (-) |
| 4 | - | 49.6 | 4.31 | 0.43 | 39.65 (-) |

* The germination was 0, 10, 35, 65, 80 and 100 % at 48, 56, 72, 80, 88 and 96 h respectively. Therefore, lag was 49.0 h and the rate (1/h) was 0.013.

** Residual variance exceeded the variance of cumulative germination.

Germination occurred rapidly at around 22 °C and was progressively delayed as temperatures diverged from this temperature (Figure 1). The regression lines representing $t_{50}$ intersected at about this temperature (Figure 3).

The spread of germination was longer (Figure 1) and the lag phase (1) was shorter (Table 3) at extreme temperatures (especially at 5 °C and 3 °C than in 30.0 °C). Between these extremes the spread and the lag phase of germination were shorter. However, time taken for 10, 50 and 90 percentiles remarkably increased as temperature diverged from optimal range, especially at sub-optimal temperatures.

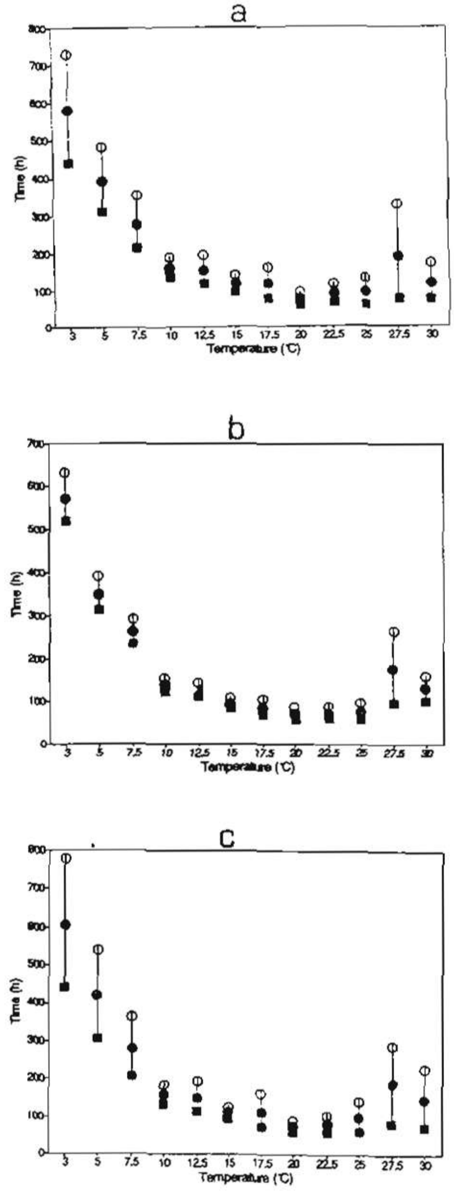


Figure 1. Time to onset (h) and spread (h) of actual germination (a) estimated with Weaver's function (b) and Weibull function (c) in response to different constant temperatures (°C). Bars represent time to 10 %, ( ), 50 % (0) and 90 % (0) germination.

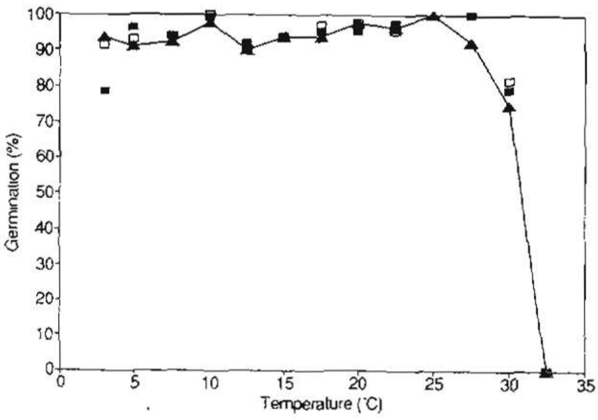


Figure 2. The effect of constant temperatures (°C) on the final percentage germination ( ) after 816 h and the estimation by Weaver (Δ) and by Weibull ( )

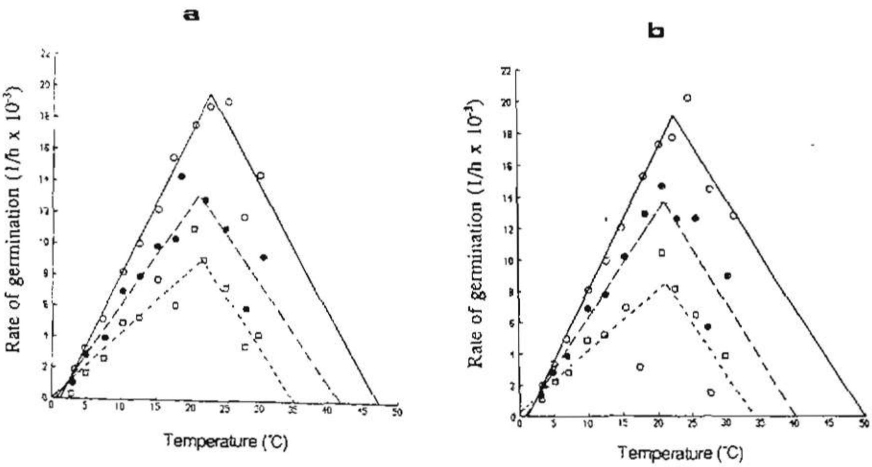


Figure 3. The effect of constant temperatures (°C) on the rate of germination (h-1) calculated with Weibull function (a) and Weaver's function (b) in relation to germination percentiles of 10 % ( ), 50 % (O) and 90 % (O).

Tablo 3. Constants Describing the Cumulative Percentage Germination Curves Derived From the Weibull and Weaver's functions at Different Constant Temperatures[a].

| Temperature (°C) | Weibull | | | | Weaver | | | |
|---|---|---|---|---|---|---|---|---|
| | A (%) | k (1/h) | l (h) | c | A (%) | d | l (h) | b |
| 3.0 | 78.6 | 0.002 | 337.9 | 2.0 | 91.4 | 4.1 | 343.0 | 5.44 |
| 5.0 | 96.3 | 0.010 | 274.7 | 1.2 | 93.1 | 2.9 | 323.6 | 4.70 |
| 7.5 | 94.1 | 0.008 | 148.0 | 2.0 | 94.1 | 3.5 | 148.6 | 4.67 |
| 10.0 | 99.1 | 0.022 | 109.6 | 1.8 | 99.9 | 3.0 | 103.1 | 3.45 |
| 12.5 | 92.2 | 0.019 | 92.1 | 1.6 | 90.9 | 2.6 | 85.3 | 3.76 |
| 15.0 | 94.2 | 0.028 | 72.9 | 1.8 | 93.8 | 3.3 | 66.4 | 3.55 |
| 17.5 | 95.3 | 0.019 | 52.0 | 1.3 | 97.1 | 2.2 | 50.0 | 3.65 |
| 20.0 | 95.5 | 0.042 | 38.0 | 3.1 | 97.5 | 2.9 | 49.4 | 2.98 |
| 22.5 | 97.5 | 0.022 | 39.9 | 1.9 | 95.4 | 3.3 | 38.4 | 3.61 |
| 25.0 | 103.1 | 0.017 | 41.4 | 1.4 | 101.8 | 2.7 | 23.0 | 4.01 |
| 27.5 | 122.4 | 0.005 | 24.2 | 2.0 | 103.0 | 2.0 | 18.3 | 5.08 |
| 30.0 | 79.4 | 0.015 | 58.4 | 1.1 | 81.7 | 2.4 | 52.1 | 4.00 |

a : each value is a mean of four replicates.

At a given percentile there was close agreement in $T_b$ and in $T_o$ calculated by the two methods (Table 4) (e.g. $T_b$ ranged between 0.1 and 0.8 °C and $T_o$ between 20.9-22.9 °C).

Both models colesely estimated $T_a$ irrespective of germination percentiles although low coefficients of determination ($R^2$ = 0.17-0.67) suggest $T_m$ was poorely defined.

There was also close agreement between the two models in the calculation of thermal time. Thermal time requirements ranged from 44.1 to 107.0 °Cd depeding on the percentile chosen (Table 4). ø, calculated by Weibul model increased from 44.1 to 96.9 °Cd as the germination percentile increased as did $ø_2$ (52.6 to 65.5 °Cd).

## DISCUSSION

Of the equations initially fitted, logistic function and Hunter's method did not edaquately describe simple parameters of the germination. They were sensitive to the estimation of initial parameters and often did not converge during the iteration

Table 4. Estimation of Cardinal Temperatures and Thermal Time Requirements (ø, and $ø^2$) (°Cd) Using Weibull and Weaver's Functions (b is the Coefficient of Regression (b) Between Germination Rate (1/h) and Temperature).

| | Temperature (°) | | | Sub-Optimal range | | | Supra-optimal range | | |
|---|---|---|---|---|---|---|---|---|---|
| Basis of fit | Base (tb) (Confidence limits) | Optimum ($t_o$) | Maximum ($t_m$) | b ($x10^{-3}$) | $ø^1$ | $r^2$ | b ($x10^{-3}$) | $ø^1$ | $r^2$ |
| Weibull function | | | | | | | | | |
| $t_{10}$ | 1.4 (0.42-2.27) | 22.6 | 47.3 | 0.944*** | 44.1 | 0.97 | -0.79** | 52.6 | 0.18 |
| $t_{50}$ | 0.8 (-0.37-2.18) | 21.0 | 41.8 | 0.657*** | 63.4 | 0.94 | -0.64** | 65.5 | 0.39 |
| $t_{90}$ | 0.1 (-2.17-3.71) | 21.3 | 35.2 | 0.430*** | 96.9 | 0.74 | -0.66*** | 63.5 | 0.67 |
| Weavers function | | | | | | | | | |
| $t_{10}$ | 1.3 (0.42-2.27) | 22.5 | 50.4 | 0.903*** | 46.1 | 0.97 | -0.68** | 60.9 | 0.18 |
| $t_{50}$ | 0.7 (-0.11-1.75) | 21.1 | 39.9 | 0.677*** | 61.5 | 0.97 | -0.73*** | 57.3 | 0.41 |
| $t_{90}$ | 0.7 (-2.87-2.60) | 21.3 | 33.9 | 0.389*** | 107.0 | 0.75 | 0-0.68*** | 61.5 | 0.51 |

* $P < 0.05$, ** $P < 0.01$, *** P ( 0.001, ns = not significant.

process. The method of Hunter et al., (1984), unlike other equations including logistic function, is based on explicit probablistic assumptions and aims to summar ise the main wave of germination, assuming that early germinating seeds are likely to have a greater planting value. Although this method may offer advantage over other equations which erraneously assume that the cumulative germination to successive time are independent this method uses the number of germinated seeds and could also identify rapidly germinating seed lots/lines. Due to problems of poor fit its flexibilty to various data must be proved. It fails also to describe all facets of the germination progress curve such as the spread and the different germination percentiles.

In view of their better description of cumulative germination and better fit to the germination data, the Weibull and Weaver's functions were superior to other functions tested, confirming previous reports (Brown and Mayer, 1988 a; Dumur et al., 1990). They allowed reconstruction of the time course of germination for comparison between different temperature treatments. However, being insensitive to the initial estimates of parameters, Weibull function gave a quicker convergence and a relatively easy fit with non-linear regression than Weaver's model.

A linear relationship between temperature and rate of 50 % germination was established in 31 vegetable species, including the grain legumes Phaseolus vulgaris l., Pisum sativum L. and Vicia faba L. (Bierhuizen and Wagenvoort, 1974). Similarly Garcia-Huidobra et al. (1982) derived an array of straight lines to describe the relationship between temperature and the respective rates of progress of germination to 10 %, 20 % .... 90 % and subsequently defined the cardinal temperatures (tb, to and tm) and the thermal time requirements for germination of pearly millet (Pennisetum typhoides S. & H.) Thus data from a range of temperatures fall on two curves at a given percentile, one at sub-optimal and other at supra-optimal temperatures. As an extension of this relationship, Ellis et al. (1986) described germination for a single seed population by two cumulative normal distribution curves, being sub and supra optimal temperatures, and subsequently estimated cardinal temperature and thermal time requirements of chick pea. Later Ellis et al. (1987), reduced the experimental temperatures to 4 points, estimated base temperature and thermal time requirements repeating probit analyses until the residual variance was minimized. Dumur et al. (1990) later suggested the Weibull function was superior to the Probit model which enhanced the estimation of the cardinal temperatures and thermal time requirements by utilizing data from the original gemination progress curves.

Base temperatures estimated for 50 % germination for Albatross (0.8 °C) were similar to that of Dumur et al. (1990) for cv Alfred (0.6 °C) and of Bierhuizen and Wagenvoort (1974) for a broad bean (0.4 °C). Optimum temperatures ($t_o$) (21.0-22.6) were close to that estimated for other cultivars of faba beans (20.5-26.0 °C) (Ellis et al., 1987; Dumur et al., 1990). Maximum temperatures ($t_m$) (33.9-50.4 °C), anomalous values apart, were also consistent with that of Ellis et al. (1987) and Dumur et al. (1990) for other cultivars of faba beans (values of 37.0 to 64 °C). But, the poor fit coupled with erratic values in the supra-optimal temperature range and the lack of actual germination beyond 30.0 °C suggest that tm may be poorly defined. This is in accordance with previous work (Ellis et al., 1987; Dumur et al., 1990) which reported $t_m$ values up to 64 °C in spite of the fact that no genmination occurred above 39-40 °C. So, $t_m$ should be interpreted with caution for physiological response to increases in temperature above the optimum range may not be linear.

Thermal time requirements for 50 % germination (ø1 and $ø_2$) were comparable to that calculated for other faba bean cultivars (52.8-120.0 °Cd) (Dumur et al., 1990; Ellis et al., 1987; Bierhuizen and Wagenvoort, 1974).

Our evidence shows that both Weibull and Weaver's functions have quick fit and sufficient parameters to provide an acurate model of the germination progress curve. Since powerful computers are widely available analysis of germination data by one of the above functions may caracterize many facets of germination and allow estimation of cardinal temperatures and thermal time requirements.

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