

Application of a Hydrothermal Time Seed Germination Model Using the Weibull Distribution to Describe Base Water Potential in Zucchini (*Cucurbita pepo* L.)

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Abstract Seed germination has been modeled extensively using hydrothermal time (HTT) models. Generally, a normal distribution for base water potential $\psi_{b(g)}$ and a linear upward shift in $\psi_{b(g)}$ with increasing temperature were used within these models to relate variation in $\psi_{b(g)}$ to the variation in time to germination of a given fraction of seeds. However, little research has tested these assumptions. Therefore, the main purpose of this study was to test the assumption of normality in the distribution of $\psi_{b(g)}$ by germinating zucchini (*Cucurbita pepo* L.) seed with non-dormant seed across a range of constant ψ at sub- and supra-optimal temperatures. Zucchini seeds were incubated at six constant temperature regimes (15, 20, 25, 30, 35, and 37 °C) at each of the following osmotic potentials (0, -0.15, -0.30, -0.45, and -0.60 MPa). Results indicate that when incorporated into an HTT model, the Weibull distribution ($R^2 = 0.85$; RMSE = 11.89) more accurately described $\psi_{b(g)}$ and germination course over sub- and

supra-optimal temperatures than HTT based on a normal distribution ($R^2 = 0.77$; RMSE = 14.80). According to these models, base temperature and base water potential for the 50th percentile ($\psi_{b(50)}$) were estimated at 9.38 °C for both models and -1.27 and -1.31 MPa by the normal and Weibull distribution, respectively. Given the flexibility of the Weibull distribution this model not only provides a useful method for predicting germination but also a means for determining the distribution of $\psi_{b(g)}$.

Keywords Distribution · Modeling · Seed germination · Normal · Zucchini · Weibull

Introduction

Cucurbita pepo L. is an important species of vegetable food crop (family *Cucurbitaceae*, the gourd or cucumber family) endemic to the New World and includes many common kitchen squash varieties such as acorn squash, pumpkin, zucchini, and patty pans (http://eol.org/data_objects/26504993). Archeological evidence shows it to be one of the world's earliest domesticated species, first cultivated between 8,000 and 10,000 years ago in Mesoamerica, probably southern Mexico (Decker 1988). Most publications pertaining to molecular studies of *Cucurbita* sp. involve biomass, age and/or fruit storage period, but very few reports assess the application of mathematical models to describe seed germination (Decker-Walters and others 2002; Loy 2000; Gardingo and others 2009). Zucchini (*Cucurbita pepo* L.) is an important vegetable food crop with medicinal value, including treatment for benign prostatic hyperplasia and leprosy (Hamissou and others 2013), that is consumed either raw in salads or cooked in soups.

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Seed germination, which is an important stage in the life history of a plant, affects seedling development, survival, and population dynamics (Wang 2005). Environmental factors regulating germination include temperature, water, and oxygen for non-dormant seeds, and light and chemical environment for dormant seeds (Baskin and Baskin 2001). Temperature (T) and water potential (ψ) mainly drive seed germination rate (GR) when aeration is not restrictive for non-dormant seeds (Bradford 2002). These two factors have been successfully combined in hydrothermal time (HTT) models to describe the time course of germination for a wide range of plant species, at both sub- and supra-optimal temperatures (Watt and others 2010, Watt and others 2011). Generally, the distribution of germination time can be expressed mathematically as (Gummerson 1986; Bradford 1995):

$$t_g = \theta_{HT} / [(\psi - \psi_{b(g)})(T - T_b)], \tag{1}$$

where θ_{HT} is the hydrothermal time constant, $\psi_{b(g)}$ is the base water potential threshold for the fraction of the seed population that germinates, g , T_b is the base temperature at which seeds can germinate, and t_g is the time required (days) for a fraction or percentage, g , of the seeds to germinate. In this model (Eq. 1), hydrothermal time (θ_{HT}) and T_b are assumed to be constant for a seed population, whereas ψ_b varies for different seed fractions, g , usually following a normal distribution (Gummerson 1986; Bradford 1990), which can be modeled using the following equation:

$$\psi_{b(g)} = \psi_{b(50)} + \text{probit}_{(g)}\sigma\psi_b, \tag{2}$$

where $\psi_{b(50)}$ is the 50th percentile of the base water potential distribution, $\text{probit}_{(g)}$ is the probit function that calculates the standard normal deviation for a specified cumulative probability in a normally distributed population ($= g$), and $\sigma\psi_b$ is the standard deviation of ψ_b values in the population. The HTT model is usually fitted to germination data by substituting the right hand side of Eq. 2 for $\psi_{b(g)}$ in Eq. 1 and rearranging the resulting equation in terms of probit as

$$\text{Probit}_{(g)} = [\psi - (\theta_{HT}/(T - T_b)t_g) - \psi_{b(50)}] / \sigma\psi_b. \tag{3}$$

Gummerson’s HTT model does not account for thermoinhibition (that is, the decline in germinability within a seed population as temperatures increase above the optimum for germination) because it assumes that all parameters are independent of T and ψ . To be able to calculate thermoinhibition in the HTT model, Alvarado and Bradford (2002) and Rowse and Finch-Savage (2003) proposed an increase in seed ψ_b at supra-optimal temperatures. This increase in seed ψ_b reduces the rate of HTT accumulation at a specific ψ and thus decreases the GR . The increase in

seed ψ_b at supra-optimal temperatures is modeled as a simple rightward shift in the location of the normal distribution for $\psi_{b(g)}$ (Alvarado and Bradford 2002).

Alvarado and Bradford (2002) proposed that a modified HTT model can describe and predict both the timing and percentage of germination across all constant temperatures and ψ at which germination can occur according to:

$$\theta_{HT} = [\psi - \psi_{b(g)T_o} + (k(T - T_o))] / (T_o - T_b)t_g, \tag{4}$$

or

$$\text{Probit}_{(g)} = (\psi - \{\theta_{HT} / [(T - T_b)t_g]\} - \psi_{b(50)} + k(T - T_o)) / \sigma\psi_b, \tag{5}$$

where $\psi_{b(g)T_o}$ is the $\psi_{b(g)}$ distribution at T_o and represents the uncorrected base water potential for that percentile, k is a positive constant (that is, the slope of the relationship between $\psi_{b(g)}$ and T in the supra-optimal range of T). This equation adjusts $\psi_{b(g)}$ to higher values as T increases above T_o and stops the accumulation of thermal time at a value equivalent to that accumulated at T_o . Thus, temperatures above T_o do not contribute additional thermal time in the supra-optimal range.

The assumption of normality underpinning the $\psi_{b(g)}$ distribution in the original HTT model (Eqs. 1 and 2) may not be universally valid. Watt and others (2010) indicated that within the suboptimal T range, $\psi_{b(g)}$ for *Pinus radiata* D. Don and *Buddleja davidii* Franch. has a skewed pattern best described by the Weibull distribution (Weibull 1951) that converges to a closed minimum value of $\psi_{b(g)}$, rather than an open left limit, as described by the normal distribution. Despite the wide use of HTT models to simulate germination, little research has critically examined the assumption that $\psi_{b(g)}$ within these models is normally distributed. However, at supra-optimal temperatures, germination may be very rapid for the first few seed percentiles, with no evidence of thermoinhibition (Hills and van Staden 2003), but germination for later percentiles usually would decrease with increasing temperatures. This response can be accommodated by the Weibull distribution of $\psi_{b(g)}$, but not by the normal distribution (Watt personal communication).

The normal distribution is not necessarily the best function for $\psi_{b(g)}$ in HTT models, and the ‘best’ distribution may vary with species (Watt and others 2010, Watt and others 2011; Mesgaran and others 2013). An alternative to the normal distribution that can fit a range of distribution types is the Weibull distribution. Using the Weibull distribution, $\psi_{b(g)}$ in the suboptimal T range can be modeled by the percent point function (or inverse of the cumulative distribution function), given as (Watt and others 2010):

$$\psi_{b(g)} = \mu + \alpha(-\ln(1-g))^{1/\gamma}, \quad (6)$$

where the parameter μ describes the location of the lowest value of $\psi_{b(g)}$ (that is, $\psi_{b(0)}$), γ is the shape parameter, and α is the scale parameter. Substitution of this equation for $\psi_{b(g)}$ into the suboptimal HTT model rearranged in terms of germination percentile yields:

$$g = 1 - \left\{ \exp\left[-\left(\left\{\psi - \left\{\theta_{HT}/[(T-T_b)t_g]\right\} - \mu\right\}/\alpha\right)^\gamma\right] \right\}. \quad (7)$$

The study had two key objectives. First, using data obtained from zucchini germination trials, HTT models were fitted that use the Weibull and normal distribution to describe $\psi_{b(g)}$ over a range of constant ψ and temperatures to test for normality. Second, the accuracy and bias of these two HTT models in predicting germination percentage across a range of temperatures were assessed. By achieving these two objectives, and based on parameters obtained in this study, zucchini seed germination can be modeled.

Materials and Methods

Germination Test

Seeds of zucchini (*Cucurbita pepo* L. var. F1-Hybrid) plants were provided by Pishgaman Sanat va Bazr (Tehran, Iran) in 2012. The seeds were stored in a black plastic bag and were kept in the dark at 4–5 °C before use. The seed populations used in this study were not dormant and achieved nearly complete (99 %) germination under optimum conditions. The seeds were incubated in the dark at six constant temperatures (15, 20, 25, 30, 35, and 37 °C) at each of the following five ψ s (0, –0.15, –0.30, –0.45, and –0.60 MPa), in all permutations. A ψ of 0 MPa was obtained using distilled water. Negative ψ values were obtained using polyethylene glycol (PEG 6000, Merck, Germany) solutions of various concentrations, following the Michel and Kaufman (Michel and Kaufmann 1973) equation. The ψ values of the solutions were measured using a vapor pressure osmometer (Model 5100C: Wescor Inc., Logan, UT, USA) and corrected for the effect of temperature (Michel and Kaufmann 1973). Hence, different PEG concentrations were used at 15–37 °C to obtain equivalent ψ values. Three replicates of 30 seeds were germinated in 9 cm diameter Petri dishes (SUPA Medical Devices, Tehran, Iran) on two layers of Whatman grade No. 1 (9 cm diameter, UK) filter paper containing 6 ml of distilled water and solutions, that is, 30 seeds per Petri dish \times 3 dishes = 90 seeds in total. The Petri dishes (seeds) were randomly incubated in different positions within the germination incubators with ± 0.5 °C accuracy

(Mehmert Beschickung-loading-model 100-800, Germany) in the dark, except when recording germination. Seeds were observed every 5–10 h each day, depending on the temperature, and considered to have germinated when the emerged radical was at least 2 mm long. Germination experiments at all temperatures were terminated when no new germination was recorded in the three replicates of a treatment for three consecutive days.

Model Fitting

All models were fitted using the non-linear (PROC NLIN) regression procedure of Statistical Analysis System version 9.1 (SAS Institute, Cary, NC, USA, 2004). Model fitness was statistically compared with germination data using the coefficient of determination, R^2 , and the root mean square error (RMSE).

The normal and Weibull distribution HTT models were fitted to the data using Eq. 5 and 7, respectively. Briefly, for the normal distribution HTT model, Eq. 5 was fitted without the modifier $k(T-T_o)$, whereas Eq. 7 was fitted to the data for the Weibull distribution HTT model. Using suboptimal parameter values for the normal distribution (T_b , θ_{HT} , $\psi_{b(50)}$, $\sigma\psi_b$, Table 1), Eq. 5 was then fitted to the data in the supra-optimal T range to estimate k values. In fitting the Weibull distribution HTT model to the supra-optimal T range, the parameters T_b , θ_{HT} , and μ in Eq. 7 were held at values determined from the suboptimal fit (see Table 1 for parameter values). Equation 7 was modified to account for thermoinhibition by fitting the scale parameter (α) as a function of T above the optimum, using an exponential function $\{\alpha = a + b \exp[c(T-T_o)]\}$. Values of a , b , and c , and the shape parameter, γ , were empirically estimated by fitting the non-linear function to the data. Shape (γ) and location (μ) were not modified as functions of T or ψ in the model as, in contrast to the scale parameter, they showed no discernible trend with either T or ψ (data not shown).

In both the normal and the Weibull HTT models, $\psi_{b(g)}$ is a key variable influencing model performance. Using parameter values derived from these fitted models, a “virtual” base water potential was determined, from the time to germination, by rearranging Eq. 1, in terms of $\psi_{b(g)}$ as

$$\psi_{b(g)} = \psi - \left\{ \theta_{HT}/[(T-T_b)t_g] \right\}. \quad (8)$$

The difference between the virtual and predicted $\Delta\psi_{b(g)}$ was determined as Eq. 8 minus the base water potentials predicted by Eq. 2 for the normal distribution and Eq. 6 for the Weibull distribution HTT models. Residual germination was determined as actual germination minus the germination predicted by Eq. 5 for the normal distribution and

Table 1 Parameters for the hydrothermal time models, based on normal and Weibull distributions in *Cucurbita pepo* L.

	Model parameters										Model statistics	
	T_b (°C)	$\psi_{b(50)}$ (MPa)	$\sigma\psi_b$ (MPa)	θ_{HT} (MPa°Cd)	k (MPa°Cd)	μ (MPa)	γ	a	b	c	R^2	RMSE
Normal	9.38	-1.27	0.353	532.3	0.098	-	-	-	-	-	0.77	14.80
Weibull	9.38	-	-	602.9	-	-2.97	7.51	1.732	0.342	0.108	0.85	11.89

Also shown are model statistics describing the coefficient of determination (R^2) and root mean square error (RMSE), ($n = 342$ for each model)

Eq. 7 for the Weibull distribution HTT models. Plots of $\Delta\psi_{b(g)}$ against germination and residual germination against germination for both normal and Weibull HTT models were examined for bias from the 1:1 line to determine which model provided the best fit to the data.

Results

The full set of parameter values used to describe zucchini seed germination in the sub- and supra-optimal T range for the normal and Weibull HTT models are provided in Table 1. The HTT model based on the Weibull distribution of $\psi_{b(g)}$ described sub- and supra-optimal T germination data more precisely than the HTT model based on the normal distribution (Table 1). The coefficient of determination was 0.85 and 0.77 for the Weibull and normal distributions, respectively. In addition, RMSE for the Weibull distribution HTT model was 2.91 % lower than the normal distribution (14.80 versus 11.89 %) (Table 1). Using both the normal and Weibull distributions of $\psi_{b(g)}$, predicted germination, as described using the probit, indicated marked bias (Fig. 1a and b). Overprediction by these models at high and low values of probit was clearly evident in plots of actual probit against predicted probit (Fig. 1a and b), and residual probit against actual probit (Fig. 1c and d). However, there was a difference in precision between HTT models (Table 1): the HTT model based on the normal distribution was more biased than that based on the Weibull distribution. The greater bias in the normal distribution HTT model was clearly evident in the plot of actual against predicted germination (Fig. 2a), and in the plot of residual against actual germination (Fig. 2c). In comparison, the model that described $\psi_{b(g)}$ using the Weibull distribution had relatively little bias (Fig. 2b and d). Generally, the contrast between models was clearest in plots of residual against predicted germination (Fig. 2c and d). A second-order linear fitted to the residual plots and developed from the model with the normal distribution of $\psi_{b(g)}$ showed a distinctly linear function with $R^2 = 0.12$ and $P > 0.0001$. Compared to this plot, the second-order linear fitted to the residual plot, obtained from the model

with the Weibull distribution of $\psi_{b(g)}$, showed little apparent bias, with $R^2 = 0.08$ and $P > 0.0001$ (Fig. 2c and d).

In the Weibull HTT model, predicted increases in the scale parameter α with increasing temperature above the optimum temperatures showed a non-linear curve, respectively (Fig. 3). The normal distribution model for supra-optimal temperatures (Eq. 5) assumes that the increase in $\psi_{b(g)}$ as a function of increasing T is constant for all germination percentiles. However, when observed, $\psi_{b(g)}$ (Eq. 8) was plotted against germination percentile. In this case, there appeared to be very little increase in $\psi_{b(g)}$ for the earliest germinating percentiles, which had similar values at all supra-optimal temperatures (Fig. 4). The normal distribution HTT model provided a relatively poor and biased fit to the observed $\psi_{b(g)}$ (Fig. 4a). In contrast, increases in observed $\psi_{b(g)}$ with both T and germination percentiles were accurately modeled by the modified Weibull HTT model (Eq. 7). This function fitted the observed $\psi_{b(g)}$ well and, importantly, described the divergence in the observed $\psi_{b(g)}$ between contrasting supra-optimal temperatures that occur with increasing germination percentile (Fig. 4b). The probability distribution function for $\psi_{b(g)}$, as fitted by the Weibull function, over the supra-optimal T range shows a compression and a right-hand shift with increases in temperatures above T_o (Fig. 5).

Discussion

There are very few studies that investigate the germination of zucchini seeds in the academic literature (Nerson and Hazor 2008; Nerson 2009) even though other studies on other cucurbitaceous crops exist (reviewed in Nerson 2007), including for fluted pumpkin (*Telfairia occidentalis* Hook. f.) (Ajayi and others 2006), medicinal pumpkin (*C. pepo* convar. *pepo* var. *styriaca*) (Ghaderi and others 2008), cucumber (*Cucumis sativus* L.), pumpkin (*Cucurbita maxima* Duch. and *C. pepo*), watermelon (*Citrullus lanatus* L.), melon (*Cucumis melo* L.) and snake cucumber (*C. melo*) (Kaymak 2012), pumpkin (Zehtab-Salmasi 2006), and star cucumber (*Sicyos angulatus* L.) (Qu and

Fig. 1 Relationship between actual and predicted **a** and **b**, and actual and residual probit **c** and **d**. **a** and **c** are for normal distribution, while **b** and **d** are for the Weibull distribution (data using for each plot is $n = 342$)

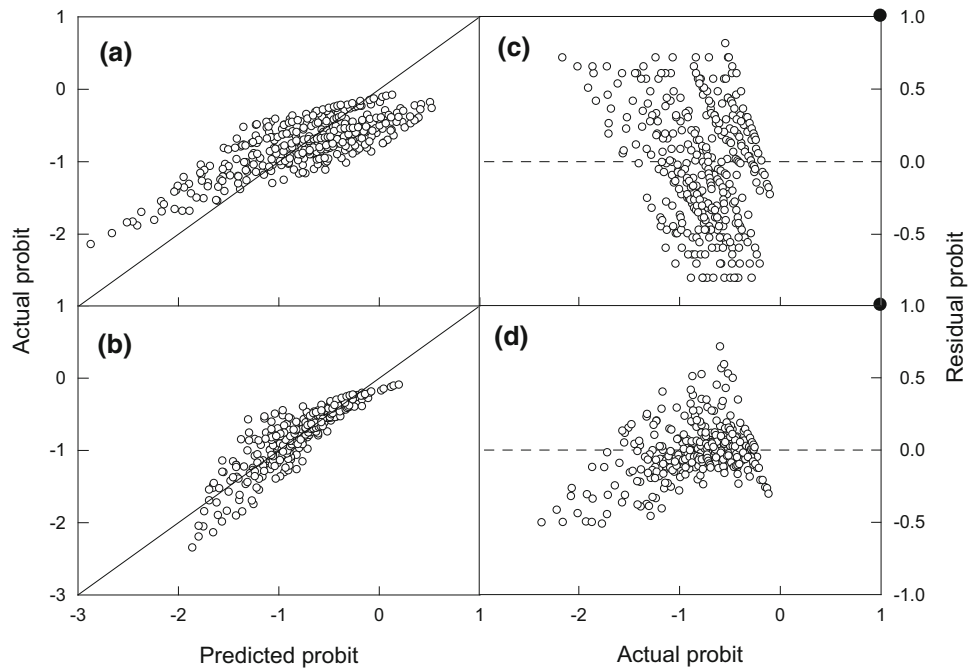
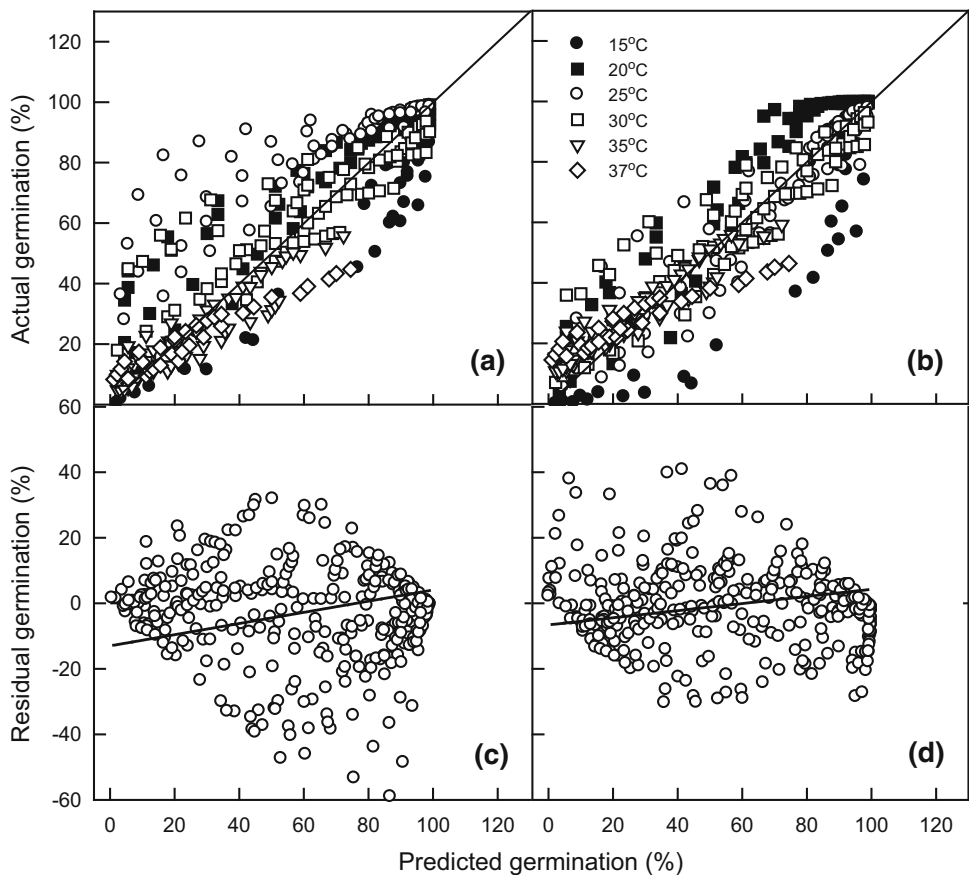


Fig. 2 Relationship between actual and predicted germination **a** and **b**, and residual and predicted germination **c** and **d**. **a** and **c** were fitted using the normal distribution, while **b** and **d** were fitted using the Weibull distribution. The linear regression model of best fit is shown in **c** and **d** (data using for each plot is $n = 342$)



others 2010). The germination of a population of zucchini seeds was modeled using the concept of hydrotime or hydrothermal time. Hydrotime analysis is considered by

many researchers to have physiologically and ecologically relevant parameters even though it is an empirical method (Alvarado and Bradford 2002; Allen, 2003). In its standard

form, it provides several useful indices of seed quality related to stress tolerance, $\psi_{b(g)}$, speed, θ_H , and uniformity, $\sigma\psi_b$, of germination (Bradford and Still 2004). However, almost all previous applications of hydrotime and hydrothermal time assumed a normal distribution for $\psi_{b(g)}$ (using probit analysis; Bradford and Still 2004; Meyer and Allen 2009). The HTT model assumes the timing, rate, and percentage of seed germination for a constant temperature to be controlled by the difference between ψ of the seedbed and the ψ_b for a given percentile (Kebreab and Murdoch 1999; Alvarado and Bradford 2002; Rowse and Finch-Savage 2003). One of the advantages of this assumption is that the parameters $\sigma\psi_b$ and mean $\psi_{b(g)}$, along with θ_{HT} and T_b , provide a complete description of the likely germination behavior of a seed population and allow for germination behavior of different species to be compared (Köchy and Tielbörger 2007). However, the assumption has not

been tested critically (see Watt and others 2010; Mesgaran and others 2013).

The normal distribution was selected for the original model on the basis of its adequacy of fit to the data in the original studies and for its mathematical convenience, rather than for any theoretical reasons (Mesgaran and others 2013). The normal distribution is symmetrical about the mean (so that mean = median = mode) and serves as a reasonable approximation to the central regions of frequency distributions (Mesgaran and others 2013). However, $\psi_{b(g)}$ is in the tail of the distribution and may thus be expected to follow (right) skewed frequency patterns (Watt and others 2010, Watt and others 2011).

Our results suggest that the Weibull distribution may be more suitable than the normal distribution for modeling $\psi_{b(g)}$ in the sub- and supra-optimal T range in zucchini. This is also clearly illustrated by our results, which show the Weibull distribution fitted $\psi_{b(g)}$ at low values of germination more accurately than the normal distribution, which markedly underpredicted $\psi_{b(g)}$, and consequently predicted actual germination to occur more rapidly than was observed (Fig. 2a and c).

Watt and others (2010) reported that the Weibull distribution provided more accurate and unbiased estimates of $\psi_{b(g)}$ and germination dynamics in hydrothermal time modeling of *Buddleja davidii* Franch. and *Pinus radiata* D. Don germination than when using the normal distribution. (Watt and others 2011) reported similar results in four unrelated species: *B. davidii*, *P. radiata*, *Allium cepa* L. cv. ‘Hyton,’ and *Daucus carota* L. cv. ‘Narman.’ (Mesgaran and others 2013) investigated eight statistical distributions (Gumbel, Weibull, Normal, Log-Normal, Logistic, Log logistic, Inverse Normal, and Gamma) in four weed species: *Hordeum spontaneum*, *Phalaris minor*, *Heliotropium europaeum*, and *Raphanus raphanistrum*. They reported that the normal distribution is not necessarily the best

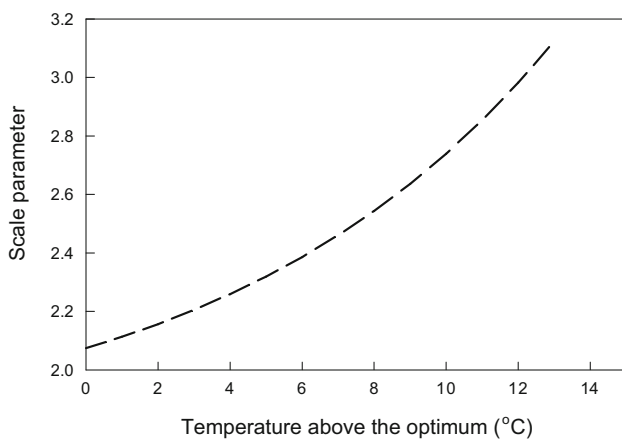


Fig. 3 Relationship between the scale parameter in the Weibull distribution (α) hydrothermal time model and the temperature above the optimum. The functional form of equation and parameter values are given in Table 1

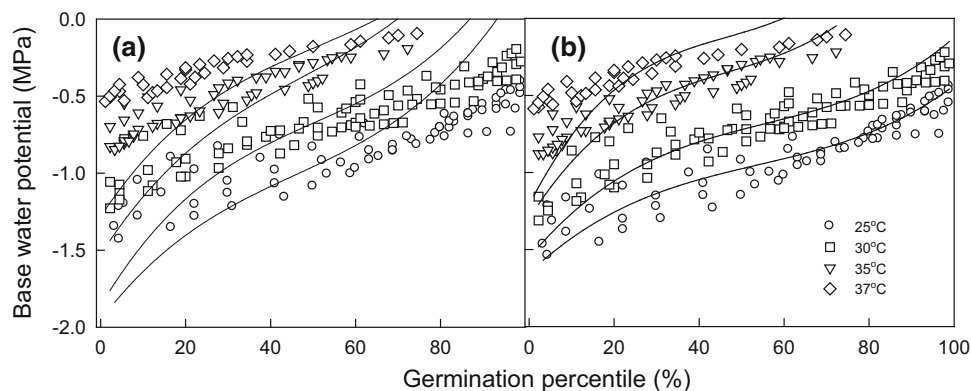


Fig. 4 Relationship between germination percentile and observed (symbols) and predicted (lines) base water potential in the super-optimal temperature range. **a** Predicted base water potential (multiple

lines) using the normal distribution hydrothermal time (HTT) model, **b** predicted base water potential (multiple lines) determined from the Weibull distribution HTT model (data using for each plot is $n = 242$)

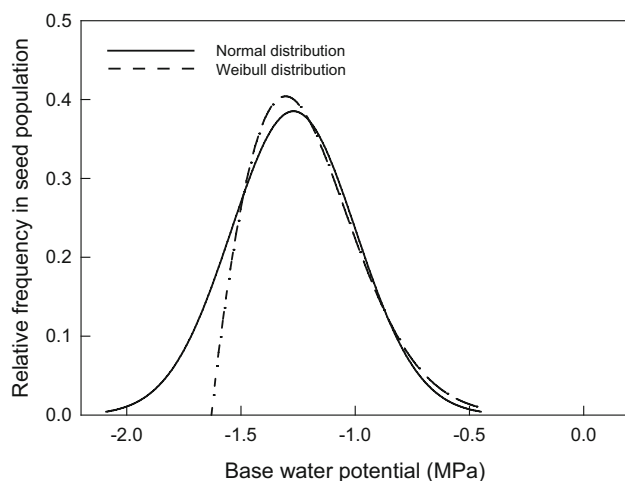


Fig. 5 Probability distribution function of the predicted base water potential for the hydrothermal time models based on the normal and Weibull distribution for *Cucurbita pepo* L. These distribution functions were generated using parameter values described in Table 1

function for $\psi_{b(g)}$ in hydrothermal models and, indeed, may give much more biased predictions than alternative functions, although the ‘best’ distribution depended on the species.

As with the normal distribution model, the parameters of the Weibull HTT model can be readily interpreted to yield information about the population frequency distribution for $\psi_{b(g)}$, enabling a comparison of the germination behavior of different seed populations. The location parameter (μ) specifies the lowest $\psi_{b(g)}$ value possible in the population $\psi_{b(0)}$. The median (M) and mode (Mo) can be readily determined by Eqs. (9) and (10), respectively, from the location (μ), scale (α) and shape (γ) parameters as

$$M = \mu + \alpha (\ln(2))^{1/\gamma}, \quad (9)$$

$$Mo = \mu + \alpha (1 - 1/\gamma)^{1/\gamma}, \quad (10)$$

if $\gamma > 1$.

The median specifies the value of $\psi_{b(g)}$ for the population, and the mode will specify the location of the peak in the probability distribution function for $\psi_{b(g)}$. The base water potential (ψ_b) for germination of the 50th percentile, $\psi_{b(50)}$, was calculated as -1.27 and -1.31 MPa by the normal and Weibull distributions, respectively. Given the flexibility of the Weibull distribution, this model is likely to be useful for determining whether this right and left skewed distribution of $\psi_{b(g)}$ is a population characteristic common to most plant species.

Consequently, the germination response of zucchini to all T_s and ψ_s can be more accurately described by the Weibull distribution than the normal distribution HTT model. The parameters of this model can be used to quantitatively characterize and compare the physiological status of zucchini seed populations under different

environmental conditions, thus indicating its usefulness as a predictive tool for zucchini germination time courses.

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