Cardinal temperatures for seed germination of three Quinoa (Chenopodium quinoa Willd.) cultivars

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ABSTRACT

Quinoa (Chenepodium quinoa Willd.) is a grain-like crop which has a high potential of crop yield under arid environments. The objective of this study was to evaluate the responses of seed germination rate and percentage to temperatures and estimate cardinal temperatures in three quinoa cultivars (i.e., Sajama, Titicaca and Santamaria). Germination of quinoa seeds were daily counted at the temperatures ranging from 5.0 to 40.0°C (5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0 and 40.0 °C). Four relevant regression models of segmented, beta, dent-like and modified beta were fit to germination rates with temperatures and subsequently the parameters including base temperature, optimum temperature, ceiling temperature and maximum germination rate were estimated. The accuracy of the model was measured by using RMSE (root mean square of error) and Aikaik Information Criteria (AIC). The interaction between temperature and cultivars effect was significant ($p \leq 0.0001$). For Sajama and Santamaria, the highest germination percentage occurred between 15-35 °C, while for Titicaca the highest germination was taken place between 5-35 °C. The beta and beta modified models for Santamaria and Sajama and the dent-like model for Titicaca were found to be the best models for predicting the thermal parameters of germination. Optimum thermal range for germination of Sajama was estimated at a wider ranges (i.e., 18-36 °C) rather than the other two cultivars of Santamaria (i.e., 23-35°C) or Titicaca (i.e., 22-35°C). The results of current study showed that quinoa is capable enough to germinate over a wide range of temperatures from 1.0 °C (T_b) to 54.0 °C (T_c).

Keywords: Beta, dent-like, germination percentage, model, thermal parameters.

تعیین دماهای کاردینال سه رقم کینوا (Chenepodium quinoa Willd)

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چکیدہ

واژههای کلیدی: درصد جوانهزنی، بتا، دندان-مانند، مدل.

Introduction

Quinoa (Chenopodium quinoa Willd.), as an autogamous plant, originated from the Andean region of South America (Ceccato et al., 2011) and belongs to the Chenopodiaceae family, a plant family comprising the highest proportion (44 %) of halophytic plant species (Flowers et al., 1986). Quinoa is currently regarded as one of the most important grain crop, mainly in terms of fatty acid composition of seed oils (Ando et al., 2002), possessing high amounts of vitamins and minerals (Comai et al., 2007), protein content and amino acid balance for human nutrition because of its high lysine and methionine levels (Bhargava et al., 2006).

On the other hand, the world is mostly being encountered with more food production with less water availability (Geerts et al., 2008). In this sense, quinoa is argued to possess a high tolerance level against frost (Jacobsen et al., 2005), drought (Garcia et al., 2003) and soil salinity, as well (Prado et al., 2000; Jacobsen et al., 2003). There are reports of high adaptability quinoa of cultivars worldwide, including arid and high rainfall areas, hot or cold climate areas, tropical areas, altitudes over 4000 m above sea level and at sea level (Bertero et al., 2004). This has increased interest in its cultivation in various regions beyond the traditional production areas inside and outside of South America. Increasing drought and water shortages in Africa and parts of Asia such as Middle East including Iran have led agricultural organizations to work on Quinoa as a potential crop under such environments.

Seed germination as a key step for plants regeneration is a complex biological process which is influenced by various environmental and genetics factors (Shafii & Price, 2001). Environmental conditions determine germination success and subsequent seedling emergence and establishment. When moisture is adequate, the germination rate of seeds is usually controlled by temperature (Kamkar et al., 2012), as a critical factor affecting onset, rate and total seed germination (Verma *et al.*, 2010). The seed germination response to temperature is a basis for models to predict the germination timing. Range of possible temperatures for germination is necessary to be determined in case that the suitability of a new region is being for introducing evaluated and cultivating a new plant (Adam et al., 2007). Cardinal temperatures [*i.e.*, base optimum (T_{o}) and ceiling $(T_{\rm h}),$ temperatures (T_c)] describe the range of temperature over which the seeds of a particular species germinate can successfully. In general, germination process increases between base and optimum temperatures, decreases between optimum ceiling and temperatures, and lastly stops beyond ceiling and less than base the temperatures (Kamkar et al., 2012). Cardinal temperatures are also important to determine the best planting date for crops (Kamkar et al., 2012).

Flores and Briones (2001) evaluated germination responses of six desert species of Cercidium praecox, Prosopis Neobuxbaumia laevigata, tetetzo, Pachycereus hollianus, Beaucarnea gracilis and Yucca periculosa to different temperatures. They reported that with increasing temperature, germination initiated earlier and the germination time (MGT) mean decreased. Jami Al-Ahmadi and Kafi (2007) studied the optimal temperatures for germination of Kochia scoparia and suggested that K. scoparia is able to adjust its germination over a wide range of temperatures, from 3.5 °C (T_b) to 50 °C (T_c), with an optimum germination temperature of 24 °C. Different types of mathematical model are used to relationship describe between germination rate and temperature. These models with biological meaning of parameters can satisfactorily predict cardinal temperatures (Soltani et al., 2006). Seed germination time of quinoa under coinciding condition of soil salinity and temperature was quantified using Weibull distribution or the loglogistic distribution (Pipper et al., 2012).

Four regression models of beta, modified beta, dent-like and segmented are being used to describe and predict germination response seed to temperature (Kamkar et al., 2012). Kamkar *et al.* (2008) also used segmented and logistic models to determine cardinal temperatures of germination in 3 millet varieties and emergence in wheat and segmented was one. describe the best To the germination rate response of Salvia leriifolia seed to temperature, three regression models, namely Intersected-Lines (ISL), Quadratic Polynomial (QPN) and Five-Parameters Beta (FPB) were used and beta model was the superior (Dashti et al., 2015). Regarding Silybum marianum L., four nonlinear regression models (i.e., segmented, beta, beta modified, and dent-like) were used at six constant temperatures describe to the germination rate-temperature relationships, with the aim of identifying the cardinal temperatures and the beta model was evaluated as the best model (Parmoon et al., 2015).

Quinoa is at attention as a crop for nutrient and industrial purposes in arid areas including Iran. Therefore, the current study was aimed to determine the response of quinoa seed germination to temperature via different models.

Materials and Methods

This study was conducted at the Seed

Laboratory of Department of the Agronomy Plant Breeding, and University of Tehran, Iran, during 2014-2015. The seeds of three quinoa cultivars (i.e., Sajama, Santamaria and Titicaca) were provided by Seed and Plant Improvement Institute, Ministry of Agriculture, Iran. The seeds were subjected to germination test in eight temperatures of 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0 and 40.0 °C in a factorial arrangement of treatments (Temperatures*cultivars). The experimental design was a completely randomized design (CRD) with four replications. Fifteen seeds of each cultivar were placed on a filter paper moistened with 5.0 mL of distilled water in petri dishes of 90 mm diameter. The seeds were incubated under fluorescent light at constant temperatures ranging from 5.0 to 40.0°C incubator in an with а temperature variation of 1.0 °C. The germination papers were moistened periodically with distilled water as required. A seed was considered as germinated if the radicle was visible about 2 mm. Germination was daily recorded for 14 days (no germination was observed after 7 days) (Prado et al., 2000).

Data of final germination became normal after an arcsin transformation and were subjected to preliminary ANOVA to find out treatment effects. Germinated seeds were counted every 24 h and cumulative germination percentage was plotted against time. From this curve, the time required to reach 50 % germination (D₅₀) was determined by fitting a logistic model of cumulative germination percentage (G) against time(t) as described by as follows (Kamkar *et al.*, 2012):

$$G = \frac{G_x}{1 + \exp[a(t-b)]}$$

Where (Gx) is the

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the maximum

germination percentage, (t) is the time required for 50 % germination (D₅₀), and both (a) and (b) are constants. The reciprocal of time to reach cumulative germination percentage to 50 % (D₅₀) of total germinated seeds was considered as the germination rate (R_{50}).

The four relevant regression models of beta, modified beta, dent-like and segmented (Table 1, Soltani et al., 2002) were fitted to R_{50} versus temperature. The parameters of models were estimated at P-values lower than standard error and the 0.05 of parameters calculated. The response of germination rates to the increasing temperature commonly follows an asymmetric peak trend. Depending on the optimum temperatures, as being a range of temperatures or a single value, the shape of model would be different. The segmented and dent-like models are being used for describing the range of optimum temperature and single value, respectively. Models beta and modified beta are used when germination rates follow a nonlinear trend with increasing temperatures.

Data transformation, ANOVA and regression analysis were carried out in SigmaPlot (11.0). Parameters were estimated via an iterative optimization method (Table 1) and standard error of parameters (SE), root mean square error coefficient (RMSE) and the of (\mathbf{R}^2) determination were used to model fit. evaluate RMSE was calculated using the following formula:

$$\mathbf{RMSE} = \sqrt{\left(\frac{1}{n}\right) \sum \left(\mathbf{Y}_{obs} - \mathbf{Y}_{pred}\right)^2}$$

Where Y_{obs} is the observed value, Y_{pred} is the predicted value and n denotes the number of points (Timmermans, 2007).

Corrected Akaike information criterion (AICc) (Burnham *et al.*, 2002) was also used to choose the most likely model:

$$AICc = AIC + \frac{2k(k+1)}{n-k-1}$$

Where AIC=2k- 2ln(SSE), SSE is the sum of square of error for the model, n denotes the total points and k is the number of parameters. The model with lower AICc value is considered as the better model.

Table 1. Beta, beta modified, segmented and dent-like models that were fitted to germination rate versus the range of temperatures (T). T_b is the base temperature, T_o is the optimum temperature, T_{o1} is the lower optimum temperature (for 3-piece segmented function), T_{o2} denotes the upper optimum temperature (for 3-piece segmented function), T_c denotes the maximum temperature, and *c* is the shape parameter for the beta function which determines the curvature of the function

Function	Formula	Reference
Beta	$\begin{split} f(T) = & \left(\frac{(T-T_b)}{(T_o-T_b)}\right) \left(\frac{(T_c-T)}{(T_c-T_o)}\right)^{\left(\frac{(T_c-T_b)}{(T_o-T_b)}\right)^c} \\ & \text{if } T > T_b \text{ and } T < T_c \\ f(T) = & \text{of } T \leq T_b \text{ or } T \geq T_c \end{split}$	Yin et al., 1995
Beta modified	$\begin{split} f(T) = & \left(\frac{(T_c - T)}{(T_c - T_o)}\right) \left(\frac{(T - T_b)}{(T_o - T_b)}\right)^{\left(\frac{(T_o - T_b)}{(T_c - T_o)}\right)} \\ f(T) = 0 \ if T \le T_b \ or \ T \ge T_c \end{split}$	Yan & Hunt, 1999
Dent-like	$\begin{split} f(T) &= (T - T_b)/(T_{o1} - T_b) \text{ if } T_b < T < T_{o1} \\ f(T) &= (T_c - T)/(T_c - T_{o2}) \text{ if } T_{o2} < T < T_c \\ f(T) &= 1 \text{ if } T_{o1} \le T < T_{o2} \\ f(T) &= 0 \text{ if } T \le T_b \text{ or } T_c \le T \end{split}$	Piper <i>et al.</i> , 1996
Segmented	$f(T) = (T - T_b)/(T_o - T_b) \text{ if } T_b < T < T_o$ $f(T) = 1 - (\frac{T - T_o}{T_c - T_o}) \text{ if } T_o \le T < T_c$ $f(T) = 0 \text{ if } T \le T_b \text{ or } T_c \le T$	Mwale et al., 1994

Results

Total Germination percentage

The results showed that seed germination percentage and seed germination rate of all the quinoa cultivars were significantly affected by temperature (P<0.0001) (Table 2).

Table 2. Analysis of variance (ANOVA) for maximum germination percentage (G_{max}) and germination rate to reach 50 % germination (R_{50}) of guinoa seed

(1t ₅₀) of quinou seed						
SOV	đf	MS				
301	ui –	R ₅₀	G _{max}			
C (Cultivar)	2	0.006^{**}	1678.476**			
T (Temperature)	7	0.004^{**}	372.994**			
T*C	14	0.0003^{**}	119.661**			
Error	72	0.00002	41.904			
CV (%)		10.51	7.27			

Germination rate and percentage for quinoa cultivars affected by the temperature range were shown in Table 3. The highest total germination percentage was observed between the ranges of 15-35 °C, while the lowest germination percentage was observed at 5.0 and 40.0 °C (Table 3). Titicaca was able to germinate at more than 88 % even at temperatures 5.0 or 40.0 °C, while for Sajama the maximum germination percentage was 80-85 % occurred at the temperatures between 15.0 to 35.0 °C. For Santamaria, the final germination percentage was 92 % occurred at temperatures between 15.0 to 30.0 °C. For all the three cultivars, there was a significant growth in germination percentage I parallel with increasing temperatures up to 40.0 °C.

For Titicaca cultivar the lowest germination rate occurred at 5.0 °C, while the highest germination rate obtained at 35°C (optimum temperature). Furthermore, for Sajama and Santamaria, the highest germination rates (0.77 and 0.41, respectively) occurred at 35.0 °C (Table 3). The results revealed the capability of quinoa cultivars to keep high germination rate in the temperatures ranging from 15.0 to 35.0 °C. This suggested high adaptability of quinoa cultivars to a wide range of environmental conditions. For Titicaca, the range of temperatures at which the highest germination rate occurred was even wider varied from 5.0 to 40.0 °C. Therefore, a wide range of temperatures are known as optimum temperatures for qunioa. Quinoa has gained worldwide attention because of its capability to grow under various stress conditions like soil salinity, acidity, drought and forest (Bhargava et al., 2006; Ceccato et al., 2011).

Table 3. Mean comparison of effects of different temperatures on seed germination rate (R_{50}) and percentage in three quinoa cultivars

Cultivals						
Cultivars	Temperature (°C)	R ₅₀ (1/day)	Germination (%)			
	5	0.010 ^j	77.33 ^{def}			
	10	0.026^{ghi}	73.33 ^{ef}			
	15	0.061 ^d	85.33 ^{bcde}			
Sajama	$20 0.072^{\circ}$		89.33 ^{abcd}			
	25 0.076 ^{abc}		96 ^{ab}			
	30	0.075^{abc}	80 ^{cdef}			
	35	0.0778^{abc}	80 ^{cdef}			
	40	0.061^{d}	77.3 ^{def}			
	5	0.011^{j}	80 ^{cdef}			
	10	0.019 ^{ij}	70.66^{f}			
	15	0.033 ^{efg}	92^{abc}			
Santamaria	20	0.033 ^{efg}	89.33 ^{abcd}			
	25	0.032^{fgh}	100 ^a			
	30	0.037 ^{ef}	92^{abc}			
	35	0.041 ^e	$68^{\rm f}$			
	40	0.022^{hi}	66.3 ^f			
	5	0.023 ^{hi}	100 ^a			
	10	0.025^{ghi}	98.66 ^a			
	15	0.057 ^d	98.66 ^a			
Titicaca	20	0.073 ^{bc}	100^{a}			
	25	0.082^{ab}	100 ^a			
	30	0.081^{abc}	100^{a}			
	35	0.083^{a}	97.33 ^{ab}			
	40	0.065 ^{cd}	88 ^{abcd}			

Determination of cardinal temperatures

For each cultivar, cardinal temperatures (*i.e.*, base, optimum, and ceiling temperatures) were estimated by fitting four regression models, as described in Table 1. For Sajama, the highest germination rate was taken place between

20.0 and 30.0 °C, demonstrating a range of optimum temperatures (Table 3). Therefore, segmented model would not be a suitable model to predict cardinal temperatures for Sajama. Parameter estimates and AICc value asserted the advantage of dent-like model over segmented one to describe germination rate with temperature and estimate cardinal temperatures parameters.





Figure 1. Germination rate of quinoa cultivars seeds as affected by increasing temperature described by dent-like model fitted to data.

Figure 2. Germination rate of quinoa cultivars seeds as affected by increasing temperature described by segmented model fitted to data.



Figure 3. Germination rate of quinoa cultivars seeds as affected by increasing temperature described by beta model fitted to data

Figure 4. Germination rate of quinoa cultivars seeds as affected by increasing temperature described by modified beta model fitted to data.

parenthesis (SE)										
Variety	Model	T _b	To	T _c	T _{o1}	T _{o2}	С	\mathbb{R}^2	RMSE	AICc
Sajama	Segmented	4(±2.5)	30(±4)	60(±24)	_	_	_	0.60	0.01	-50
	Beta	3.2(±1.3)	26.1(±4)	52.1(±2)	_	_	1.16(±2.3)	0.95	0.005	-71
	Modified Beta	2.9(±1.4)	30(±2.8)	53(±1.8)	_	_	_	0.95	0.005	-77
	Dent-like	$2(\pm 0.6)$	_	53(±11)	20(±0.53)	36.6(±3)	_	0.97	0.004	-73
Santamaria	Segmented	1(±2)	34.7(±1)	44.8(±2)	_	_	_	0.65	0.006	-69
	Beta	$1.2(\pm 1.6)$	30.2(±3)	$44(\pm 0.98)$	_	_	$0.92(\pm 1.1)$	0.83	0.004	-77
	Modified Beta	$2(\pm 2.8)$	$26(\pm 1.4)$	52(±4.7)	_	_	_	0.70	0.004	-76
	Dent-like	2(±1.9)	_	45(±7.3)	24(±2)	35(±4.5)	_	0.71	0.005	-73
iticaca	Segmented	1(±1.2)	28(±1)	58(±4.08)	_	_	_	0.90	0.008	-69.7
	Beta	$0.9(\pm 1.3)$	30.2(±16)	46(±4)	_	_	$0.86(\pm 0.4)$	0.91	0.007	-68
	Modified Beta	1(±5)	30.(±8)	51(±5)	_	_	_	0.80	0.01	-59
L	Dent-like	2.3(±1)	_	53.3(±4)	22.8(±1.06)	35.09(±2.3)	_	0.94	0.006	-74

Table 4. Estimated parameters of fitting the Segmented, Beta, Beta modified and Dent-like models to the germination rate of quinoa cultivars against increasing temperature. T_b, T_o, T_c, T_{o1}, T_{o2}, *c*, *R*² and RMSE are base temperature, optimum temperature, maximum temperature, lower optimum temperature, upper optimum temperature, and shape parameter, respectively. Standard error of estimates are shown in

Based on AICc values which were used for model selection, modified beta was chosen as the best model for estimating cardinal temperatures of Sajama cultivar (Table 4). However, base temperature (2.0 to 3.0 °C) and ceiling temperature (53.0 °C) were estimated identically with beta. modified beta and dent-like models. As the modified beta showed the best fit, its estimation for optimum temperature was accepted (30.0°C). For Santamaria, the beta model was chosen as the best model for estimating cardinal temperatures. Based on parameter estimates, the base temperature would be 1.2 (±1.01) °C. Optimal temperature was estimated as $30.2 (\pm 3)$ °C, and ceiling temperature would be 44.0 (± 0.95) °C. For Titicaca, the dent-like model showed the best fit with the lowest AICc value (Table 4). Estimated parameters showed that the base temperature would be 2.3 (± 1) °C. Optimum temperature ranged between 22 (±1.6) and 35 (±2.03) °C, and ceiling temperature was estimated as 53.3 (±4.7) °C.

Discussion

Temperature plays a critical role in the regulation of plant vital processes such as seed germination (Bare *et al.*, 1978). Each index for germination evaluation

show different responses may to germination. As shown above, for all the quinoa cultivars, germination rate susceptible was more versus germination temperature. Total in Titicaca did not vary between the temperatures of 5.0 and 35.0 °C, suggesting a wide optimum temperature range for this cultivar. Range of optimum temperature was consistently reported in the earlier literatures (Tabrizi et al., 2004), albeit, the width of optimum temperature in quinoa was highly significant.

Estimated suggested parameters significant differences in germination response to temperature among the cultivars under study. Because of higher germination rate of Titicaca and Sajama in low and high temperatures, the base and ceiling temperatures were predicted at about 2.0 and 53.0, respectively, suggesting the germination capability of these cultivars in a wide range of environmental temperatures. As a complimentary test, we made a germination test for the Titicaca and Sajama at the temperature of 46.0 °C. Germination at this high temperature is not expected to occur at high rates. For example, it has been reported that the low germination of Bidens pilosa occur at temperature 45.0 °C (Reddy et al., 1992). It has been also reported that,

Cuscuta campestris had the germination until 40.0 °C, while with increasing temperature up to 45.0 °C, the germination failed (Sarić-Krsmanović *et al.*, 2013). The relatively high germination of quinoa cultivars from 5.0 to 40.0 °C suggested the capability of quinoa to survive under cold and hot environments.

Information about cardinal germination temperatures for is important in predicting areas of distribution of plant species (Singh et al., 2008). Exposure to any temperature beyond the optimum temperature range for germination can negatively affect seed germination. Maintaining high germination rate at non-optimum temperatures suggested the survival capability of plant species in varying temperature environments (Finch-Savage et al., 2006). Base temperatures of three cultivars were in the range of 1.0 to 3.3°C, indicating that C. quinoa can germinate at low temperatures. This is an advantage for this plant species to germinate prior to other competitive species, so they would be successful in competition with neighboring plants (Kamkar et al., 2012). Such observations suggested the efficiency of cardinal temperatures using for predicting plant responses to to environmental factors. This is also used for prediction of the geographical areas where a species or genotype can germinate and establish successfully. Another interesting behavior of C. quinoa cultivars was their wide optimum temperatures. These results suggested that C. quinoa cultivars can reach their maximum germination under various environmental temperatures. An optimum condition for germination of C. quinoa could be ranged from a temperate environment (*i.e.*, 18-23 °C) to a relatively hot soil temperature (*i.e.*, 35-36 °C).

As the C. quinoa cultivars were

different in germination response to temperature, an identical model was not suitable for describing germination rate. Therefore, for each variety, an especial model was used. Saeidnejad et al., (2012) reported various responses of persicum cultivars Bunium to temperature and subsequently suggested that the difference is mostly related to the genetic disparities among cultivars. Tolyat *et al.* (2014) Furthermore. reported differences between ecotypes of Thymus daenensis in response to temperature. Regarding Silvbum marianum L., four nonlinear regression models (i.e., segmented, beta, beta modified, and dent-like) were used at six constant temperatures to describe the germination rate-temperature relationships, with the aim of identifying the cardinal temperatures and the beta model was evaluated as the best model (Parmoon et al., 2015).

Conclusions

The current study showed the differences among all the three C. quinoa cultivars in response to temperature. Therefore, for Titicaca and Sajama cultivars, the beta modified model and for the Santamaria cultivar the beta model were found more likely to describe the germination response to temperature. The main difference was found in ceiling temperature and the width of optimum temperature range. As a result, different models were used to describe specific behavior of each C. quinoa variety with increasing temperature. C. quinoa was found very tolerant to temperature, as was capable to germinate in a wide temperature Germination at very low ranges. temperatures (around 2.0 °C) and very hot conditions (>46.0 °C as observed, and more than 50.0 °C as model predicted) indicates its tolerance and abilities survival under various environmental temperatures.

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