Quantifying Cardinal Temperatures in Quinoa *(Chenopodium quinoa)* Cultivars

Cuantificación de las temperturas cardinales en cultivares de quinoa *(Chenopodium quinoa)*

González, Juan A.[1*]; Sebastián E. Buedo[1]; Marcela Bruno[1]; Fernando E. Prado[2]

[1] Instituto de Ecología, Fundación Miguel Lillo, Miguel Lillo 251, (T4000JFE) San Miguel de Tucumán, Argentina.

[2] Facultad de Ciencias Naturales e IML, Cátedra de Fisiología Vegetal, Universidad Nacional de Tucumán, Miguel Lillo 205, (4000) Tucumán, Argentina.

* Autor corresponsal: jagonzález@lillo.org.ar

➤ **Abstract** — González, Juan A.; Sebastián E. Buedo; Marcela Bruno; Fernando E. Prado. 2017. "Quantifying Cardinal Temperaturas in Quinoa (*Chenopodium quinoa*) Cultivars". *Lilloa* 54 (2). Seed germination and plant growth are affected by temperature. This study was conducted to evaluate the effect of temperature on seed germination of ten quinoa cultivars under a temperature gradient between 8 ºC and 50 ºC. The time course of germination was fitted using a logistic function. Aborted seeds and non-germinated seeds were also analyzed as function of temperature gradient. Cardinal temperatures were estimated by regression of the inverse time to 50% germination (germination rate) against temperature gradient. The minimum (T_{min}), optimum (T_{opt}) and maximum (T_{max}) temperatures for seed germination were determined using both linear (bilinear model) and polynomial (quadratic and cubic models) regressions. Based on T_{opt} estimated from bilinear and cubic models, quinoa seeds can be grouped into two subgroups: one represented by Kancolla, Chucapaca, Kamiri, Robura and Sajama cultivars with values of $T_{opt} \leq 33$ ºC, and other represented by CICA, Sayaña, Amilda, Ratuqui and Samaranti with T_{opt} values ≥ 33 ºC, respectively. Percentages of maximum cumulative germination calculated from the quadratic model were closely similar to those obtained in germination trials.

Keywords: Germination, germination models, abnormal germination, optimum temperatures.

➤ **Resumen** — González, Juan A.; Sebastián E. Buedo; Marcela Bruno; Fernando E. Prado. 2017. "Cuantificación de las temperaturas cardinales en cultivares de quinoa (*Chenopodium quinoa*)". *Lilloa* 54 (2). La germinación de las semillas y el crecimiento de las plantas son afectados por la temperatura. Este estudio fue diseñado para evaluar el efecto de la temperatura sobre la germinación de diez variedades de quinoa bajo un gradiente entre 8 ºC y 50 ºC. El curso de la germinación en función del tiempo fue ajustado utilizando la función logística. Las semillas abortadas y no germinadas también fueron analizadas en el gradiente de temperatura. Las temperaturas cardinales fueron estimadas por regresión de la inversa del tiempo de germinación al 50 % (velocidad de germinación) contra el gradiente de temperatura. La temperatura mínima (T_{min}), la óptima (T_{opt}) y la máxima (T_{max}) de germinación fueron determinadas utilizando regresiones lineales (modelo bilineal) y polinómicos (modelos cuadráticos y cúbicos). Basados en la T_{opt} estimada a partir de los modelos bilineales y cúbicos las variedades de quinoa estudiadas pueden ser divididas en dos subgrupos: uno representado por Kancolla, Chucapaca, Kamira. Robura y Sajama con un valor de T_{opt} de ≤ 33 ºC, y otro representado por CICA, Sayaña, Amilda, Ratuqui y Samaranti con una $T_{opt} \geq 33$ ºC respectivamente. Los porcentajes de germinación máxima obtenida a partir del modelo cuadrático utilizada fueron muy cercanos a aquellos obtenidos en las pruebas de germinación.

Palabras claves: Germinación, modelos de germinación, germinación anormal, temperatura óptima.

Recibido: 27/12/16 – Aceptado: 07/08/17

INTRODUCTION

Seed germination is affected by different environmental factors, being the temperature one of the most important to reach the successful germination (Bewley and Black, 1994). Temperature influences seed germination through its impact on both physicochemical processes (e.g. water uptake) and metabolic processes (e.g. enzyme-catalyzed reactions). At low temperatures enzymes are unable to adopt the active conformational state to catalyze reactions, while at high temperatures they are precipitated and cannot catalyze reactions (Dixon and Webb, 1979). Germination responses of a given seed fraction to temperature can be characterized in terms of cardinal temperatures: minimum or base temperature (T_{min}), defined as the temperature below which germination rate is zero, optimum temperature (T_{opt}), defined as the temperature at which germination rate is maximal, and maximum temperature (T_{max}), defined as the temperature above which germination rate is zero (Yan and Hunt, 1999). Cardinal temperatures are species-specific or cultivar-specific and even species origin- or cultivar origin-specific (Kamkar *et al*., 2012). Hence cardinal temperatures can be considered as important factors to predict germination performance of new cultivars and/or provenances of a determined crop in a particular environment.

Quinoa (*Chenopodium quinoa* Willd.) has high tolerance to extreme conditions such as salinity, drought, low temperatures and high solar radiation (Risi and Galwey, 1984; González *et al*., 2015). Its grain contains high quality protein with a good provision of minerals (Prado *et al.,* 2014) and better amino acid profile (González *et al*., 2011) than other crops such as wheat, rice, barley and maize (Abugoch James, 2009; Vega-Gálvez *et al*., 2010). According to FAO nutritional reference values, quinoa is considered today as a promissory alternative crop, especially for marginal regions where the growth of traditional cereals and legumes are limited (Choukr-Allah *et al*., 2016). In South America the growth range of quinoa extends from sea level in Chile and lowland regions of Peru to over 3800 m altitude in highland regions of Bolivia and Ecuador (Bazile *et al.,* 2013). Depending on genotype characteristics and phenological growth stage, quinoa can tolerate a wide range of temperature (from –8 °C to 38 °C) and relative humidity conditions (from 40% to 88%) (Jacobsen *et al*., 2005; Bazile *et al*., 2013). Seed germination and seedling development of quinoa cultivars are influenced by environmental conditions, and are strongly dependent on temperature (Jacobsen and Bach, 1998; Bertero, 2001; Bois *et al.,* 2006). González and Prado (1992) found that low germination percentages of seeds of the Sajama cultivars could be explained by an interactive effect between low temperature and soil salinity. In addition, Jacobsen and Bach (1998) reported that maximum germination percentage of the Olav cultivar occurs between 30 °C and 35 °C. This temperature range is significantly higher than the temperature range recorded in the Bolivian Altiplano during quinoa seedtime (Bois *et al.,* 2006). Beyond the body of knowledge on quinoa physiology, the effect of temperature on germination of major commercial cultivars still remains unclear. Germination response tends to be linear in a limited range of temperatures. In most cultivated species the linear range of temperatures varies between 10 and 30 °C (Bonhomme, 2000), but at extreme thresholds of both low and high germination temperatures the germinative response tends to be curvilinear (Hardegree, 2006). In this way, the estimation of cardinal temperatures can be made through linear and curvilinear models as long as limitations intrinsic to each model are recognized. The aim of this work was to analyze the effect of constant temperatures, on germinative traits of ten quinoa (*Chenopodium quinoa* Willd.) cultivars in order to determine: i) differences in cardinal temperatures determined by curvilinear models (second-and third-order regression equations) and bilinear model (linear regression equation), and ii) intracultivar variability in the percentage of germinated seeds, aborted seeds and non-germinated seeds at different temperatures. Alternative temperatures experiment was not considered according the results obtained by Strenske *et al.*, (2017). Because the estimation of cardinal temperature is highly dependent on the statistical model, bilinear and curvilinear regressions (White *et al.*, 2015) were used.

MATERIALS AND METHODS

Seed material

Seed of 10 quinoa genotypes (Amilda, Chucapaca, CICA, Kamiri, Ratuqui, Robura, Sajama, Samaranti and Sayaña), representative of the most common available commercial cultivars, were evaluated in this study. Seeds were collected from plants grown during the 2014-2015 growing season at Encalilla Experimental Station (Amaicha del Valle, Tucumán, Argentina, 22°31'S latitude, 65°59'W longitude and 1980 m asl). After harvest, the seeds were bulked, cleaned manually, placed in paper bags, and stored at 6 °C until the beginning of experiments.

Germination tests

Seeds were surface-sterilized in a 2% sodium hypochlorite solution for 2 min, washed twice with distilled water and dried on filter paper. Sterilized seeds were placed on filter paper in 5 cm glass Petri dishes (50 seeds per dish). Petri dishes were added with 2 mL of distilled water and transferred to temperature-controlled cabinet under darkness condition and relative humidity of 70%. Selected temperatures were 8 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C, and 50 °C.

In lab, germinated seeds (radicle protrusion ≥ 2 mm in length) were counted every 2 h during a 24-h period and cumulative germination percentage was plotted against time. We chose 24 h as maximum germination period because under laboratory conditions the total germination process of quinoa seeds occurs in a very short time period (~12-14 h) (González and Prado, 1992). Aborted seeds (hypocotyl emergence without radicle protrusion) (Prado *et al.,* 2000) were also counted. Seeds without radicle and/or hypocotyl emergence after a 24-h incubation period were considered as non-germinated seeds. For each cultivar and each temperature five 50-seed replicates were done.

From germination curves, the time to 50% germination was determined by fitting a logistic model of cumulative germination percentage (G) against time (t), as described by Eq. 1 and 2 (Covell *et al.,* 1986; Dumur *et al.*, 1990):

$$y = \frac{G}{1 + be^{-at}} \qquad \text{Eq. (1)}$$

$$t = \ln \frac{(G - y) / (y \cdot b)}{- a} \qquad \text{Eq. (2)}$$

Where,

y: germination percentage in each measured time;

e: base of natural logarithm;

t: time to each germination percentage;

a and b: regression coefficient constants.

Field temperature measurement

The choice of the mentioned temperature range was made according to the time that quinoa is sown in Argentinean Northwest. Normally quinoa is sown in November. So field temperature data (air and soil) were obtained for this month by a device that was put in a place where quinoa usually was grown in Tucumán province (Encalilla, Amaicha del Valle). Temperature was recorded each hour, during November 2014, using a thermocouple with temperature range between -20 ± 1 ºC and +70 ± 1 ºC (Hobo H8 RH/Temp family Data Logger; Onset Computer Corp., Bourne, MA, USA). Soil and air temperature were registered at -2 cm and 150 cm respectively.

Cardinal temperatures

Since the estimation of cardinal temperatures is highly dependent on the statistical model used to describe the germination process (White *et al.*, 2015), both bilinear and curvilinear models were used to estimate minimum (T_{min}), optimum (T_{opt}), and maximum (T_{max}) temperatures by regressing the germination rate (GR), calculated as the inverse of time to reach 50% germination, against the temperature gradient. In the bilinear model Eq. 3 and 4 were employed to describe the response of germination to suboptimal and supraoptimal temperatures:

$$y = a_1 + b_1x \ (x < T_o) \qquad \text{Eq. (3)}$$
$$y = a_2 + b_2x \ (x > T_o) \qquad \text{Eq. (4)}$$

where; a_1, b_1, a_2, and b_2 are regression parameters from which the three cardinal temperatures can be derived. Germination rate (GR) is the y-value and T_{opt} is the x-value of the breakpoint between the two lines (Kakani *et al.,* 2002). In the curvilinear model Eq. 5 and 6 were used in order to model and accurately determine cardinal temperatures:

$$y = a + bx + cx^2 \qquad \text{Eq. (5)}$$
$$y = a + bx + cx^2 + dx^3 \qquad \text{Eq. (6)}$$

where; a, b, c, and d are intercept, first, second and third-order coefficients, respectively. T_{min} and T_{max} were determined by lower and upper points were regression curve intersect the x-axis. The peak of regression curve was deemed as T_{opt} (White *et al*., 2015).

Difference between T_{max} and T_{min}, known as temperature adaptability range (TAR) (Seepaul *et al.,* 2011), showed the germination ecological range of cultivars:

$$TAR = T_{max} - T_{min}$$

Regression analysis of cumulative aborted seeds and cumulative non-germinated seeds

Based on results of ANOVA analysis, second-order regression equations to describe both the percentage of maximum cumulative aborted seeds and maximum cumulative non-germinated seeds as function of the temperature gradient, were established for all cultivars (SAS, 2010).

Statistics

Data are means of three independent experiments. To detect differences in evaluated parameters (cumulative germination percentage, cumulative aborted seed percentage and non-germinated seed percentage) among cultivars, data were processed by analysis of variance (ANOVA) and means were compared using the Tukey's test at $P < 0.05$.

RESULTS

Air and soil temperature

According to our field measurements (Encalilla, Amaicha del Valle, Tucumán, Argentina at 1,995 m asl) maximum soil temperature, registered during November 2014, at – 2 cm, was 50,7 º C and minimum one was 8.6 º C (Fig. 1). Low temperatures can induce inhibition in the germination of quinoa seeds due to embryo death as demonstrated by Rosa et al (2004). It is known that protein synthesis and activation is affected and seed reserves start to deteriorate (Bove *et al.*, 2001). On the other hand, stress induced by high temperature increases abnormal germination in many crop species (Pineda Mejia, 1999). Soil temperature may be an important data to take into account because water uptake by seeds is a function of temperature (Sigstad and Prado, 1999) (see discussion).

Germination time course

The germination time course of quinoa seeds shows typical sigmoidal curve with a triphasic pattern: phase I between 0 h and 4-6 h, phase II between 4-6 h and 8-10 h, and phase III between 8-10 h and 16 h (Fig. 2). In general, all cultivars showed a similar pattern of cumulative germination with a fairly constant percentage between 20 °C and 35 °C. Below 15 °C and above 45 °C the germination was sharply reduced. Over 50% of maximum germination was reached by all cultivars between 20 °C and 40 °C, but in Sajama and Samaranti cultivars values higher 50% of maximum germination were found between 15 °C and 45 °C. Maximum

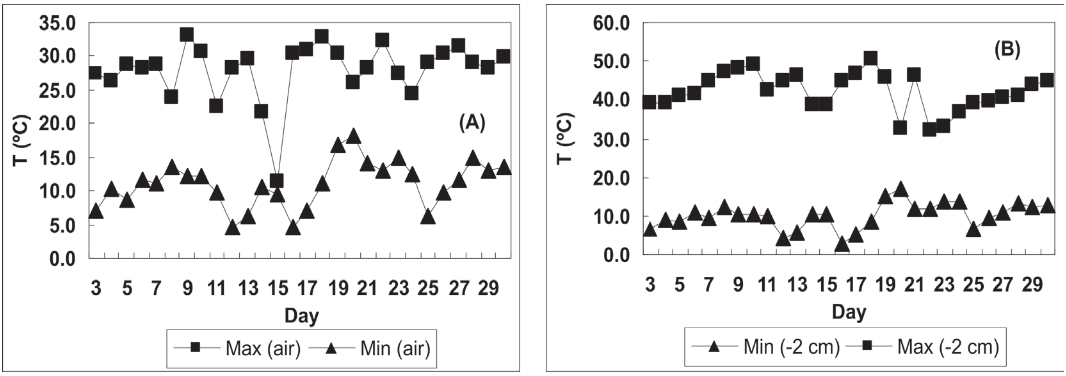

Fig. 1. Air and soil temperature (A and B respectively) in Encalilla (1995 m asl, Tucumán, Argentina) during November 2014. (Max): maximal daily temperature, (Min): minimum daily temperature.

cumulative germination was achieved during the phase III and ranged between 72.5% (Kancolla) and 90.8% (Chucapaca) (mean = 84.1%).

Maximum seed germination response to temperature

Among linear and polynomial regressions used to analyse the effect of temperature on cumulative germination, the quadratic model best described the response of maximum cumulative germination to temperature (mean R^2 = 0.927) (data not shown). Maximum germination values derived from quadratic model ranged between 68.3% (Kancolla) and 92.5% (Chucapaca) (mean = 83.3%), while those derived from bilinear model fluctuated between 78.3% (Kancolla) and 100% (Chucapaca and Sajama) (mean = 92.2%) (Table 1). Maximum cumulative aborted seeds and non-germinated seeds are shown in Fig. 3. Aborted seeds strongly increased at highest temperatures, being significantly higher in Robura cultivar with a maximum value of 53% at 50 °C. High percentages of aborted seeds were also observed in Samaranti, Kamiri, Sayaña and Ratuqui cultivars. The lowest percentage of aborted seeds (8%) occurred in Kancolla cultivar. High values of the aborted seeds/germinated seeds ratio (A/G) were observed at low and high temperatures, but were significantly higher in these last (data not shown). Non-germinated seeds also occurred along the temperature gradient. The percentage of non-germinated seeds was significantly lower than aborted seed percentage in all cultivars. Maximum percentage of non-germinated seeds was 28% and occurred in Sajama cultivar at 50 °C. Based on second-order regression equations, positive and significant curvilinear relationships between temperature gradient and both aborted seeds and non-germinated seeds were found in all cultivars. Values of R^2 ranged between 0.94 (CICA) and 0.98 (Amilda and Kamiri) for aborted seeds and between 0.84 (Ratuqui) and 0.97 (Sajama) for non-germinated seeds.

Temperature adaptability range (TAR)

The value of temperature adaptability range derived from quadratic model varied between 47 ºC (Kancolla) and 56.6 ºC (Samaranti) (mean = 53.7 ºC), whereas TAR value derived from cubic model fluctuated between 52 ºC (CICA) and 58.7 ºC (Samaranti) (mean = 55.7 ºC). Temperature adaptability range derived from bilinear model ranged between 63 ºC (Kancolla) and 67.1 ºC (Ratuqui) (mean = 65 ºC), higher than those estimated from both quadratic and cubic models (Table 2).

Cardinal temperatures

Quadratic and cubic models (second- and third-order polynomial regressions)

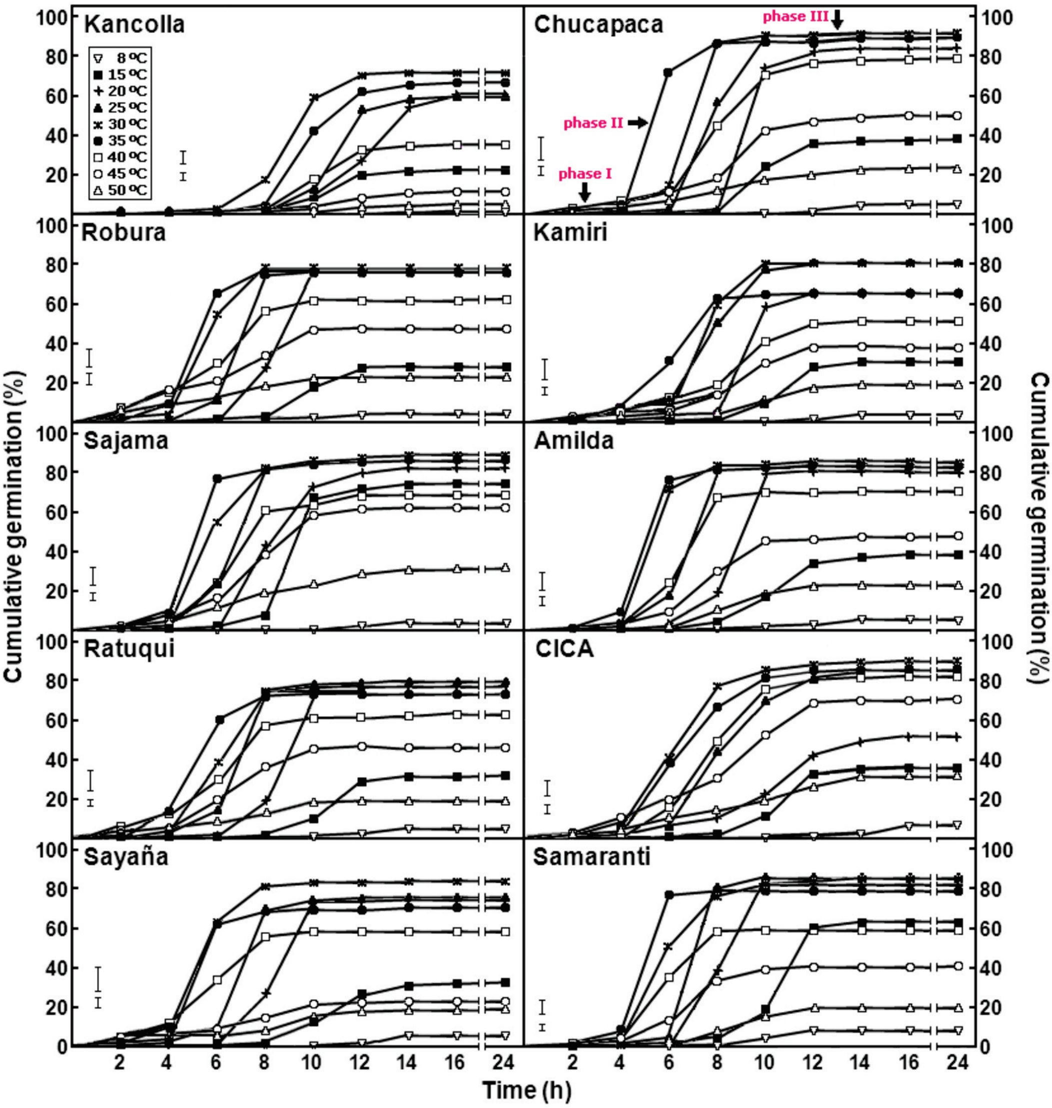

Fig. 2. Cumulative germination of 10 quinoa cultivars at different times. Values are means ± standard error of 5 replicates (250 seeds altogether). For each cultivar large and short bars represent maximum and minimum values of SE.

and bilinear model (linear regression), used to estimate minimum, optimum and maximum temperatures for germination of quinoa cultivar seeds, are shown in Fig. 4 and 5. Minimum temperatures for seed germination derived from quadratic and cubic models varied between 4.2 ºC (and Samaranti) and 5.8 ºC (Kancolla and CICA) for the former and –2.2 ºC (Sayaña) and 0 ºC (Amilda) for the latter, respectively (Fig. 4 and 5). The minimum temperature derived from the bilinear model fluctuated between –2 ºC (Sajama) and –0.2 ºC (Sayaña) (Fig. 6 and Table 3). Minimum temperatures obtained from both cubic and bilinear models are correlated to remarkable adaptation of quinoa to harsh climatic conditions of the Andean regions (Bois *et al.,* 2006). Optimum temperatures estimated from the quadratic model varied between 29.8 ºC (Kancolla) and 32.5 ºC (Ratuqui) and from the cubic model between 32 ºC (Kancolla) and 36 ºC

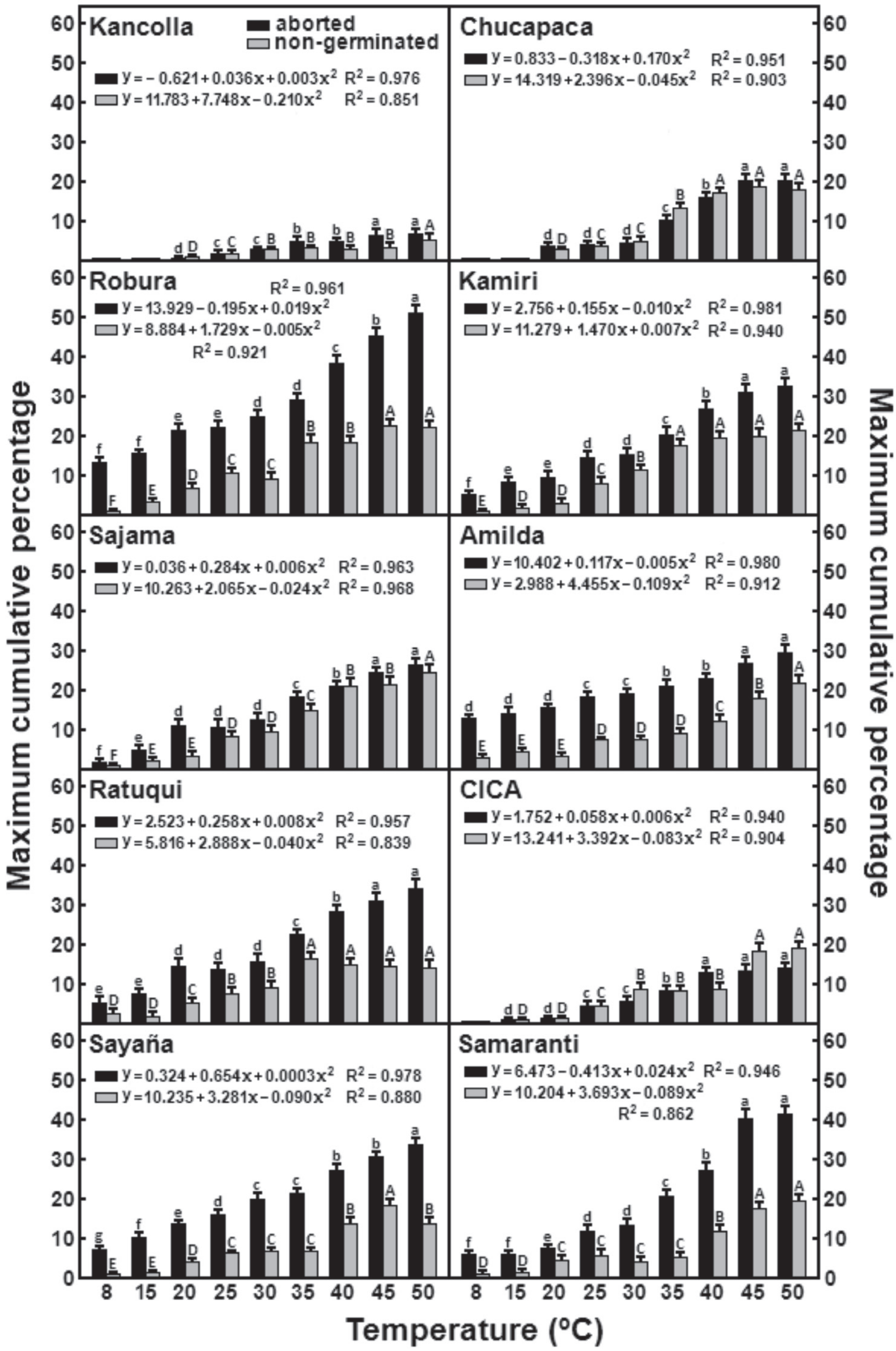

Fig. 3. Maximum cumulative aborted seeds and non-germinated seeds of 10 quinoa cultivars, at different temperatures with the fitted quadratic equations. Different lowercase letters on bars denote significant differences in maximum cumulative values of aborted seed. Different uppercase letters denote significant differences in maximum cumulative values of non-germinated seeds. Values are means ± standard error of 5 replicates.

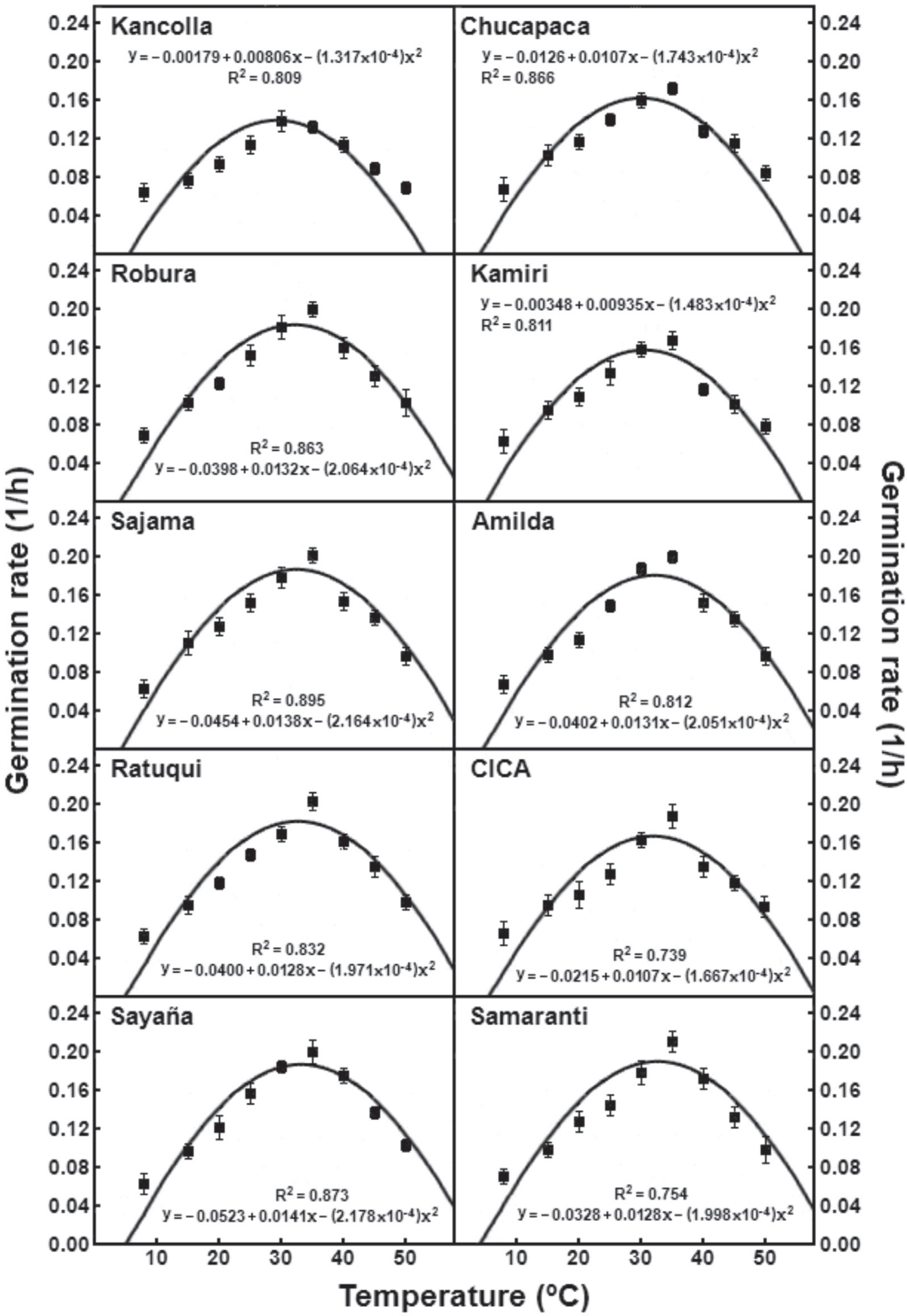

Fig. 4. Germination rate of ten quinoa cultivars incubated at different temperatures with the fitted quadratic equations. Points are the observed data, solid lines are quadratic models. Error bars represent one standard error of mean.

(Samaranti). The optimum temperature, estimated from the intercept of sub and supraoptimal temperature-response functions (bilinear model), ranged between 31.8 ºC (Kamiri) and 34.7 ºC (Amilda), slightly lower than the values derived from the cubic model. Maximum temperatures ranged between 52.9 ºC (Kancolla) and 61 ºC (Sayaña), and between 51.5 ºC (CICA) and 56 ºC (Robura and Samaranti) for quadratic and cubic models, respectively. The maximum temperature estimated from the bilinear model varied between 61.2 ºC (Kancolla and Kamiri) and 65.7 ºC (Robura), slightly higher those obtained from polynomial regressions (Table 3).

As a first approximation and based on optimum temperatures derived from cubic and bilinear models, two groups of quinoa seeds can be distinguished: one represented by Kancolla, Kamiri, Robura and Sajama cultivars with T_{opt} values between 31.8 ºC and 33.2 ºC and other represented by CICA, Sayaña, Amilda Ratuqui and Samaranti with T_{opt} values between 33 ºC and 36 ºC, respectively. No clear segregation was observed between T_{opt} values derived from quadratic model. Probably a more detailed experiment is necessary to give a conclusion in relation to these points.

DISCUSSION

Germination is of great importance because represents the first step in the plants' life. Crop success has been ascribed, among other factors, to high germinative fitness of seeds, strongly temperature dependent (Nonogaki *et al.,* 2010). Most of cereal crops exhibit maximum germination between 20 ºC and 30 ºC, but at lower and higher temperature germination values significantly decrease. In fact, longer exposure of seeds to sub- and supraoptimal germination temperatures may lead to increased exposure to soil pathogens, which can cause decrease of seedling emergence (Berti and Johnson, 2008). In contrary, crops with broader range of germination temperature can utilize soil minerals earlier compared with other species (Luna *et al.,* 2012), and then will have better opportunities to grow and develop in different agroecological regions. Our results showed that, seven quinoa cultivars exhibit their highest germination percentages between 20 ºC and 40 ºC whereas two cultivars (Sajama and Samaranti) also showed high germination percentages between 15 ºC and 45 °C. The quadratic model was significantly more accurate than the bilinear model to explain relationships between temperature and cumulative germination for all quinoa cultivars. Furthermore the predicted maximum cumulative germination percentages from the bilinear model were higher than those predicted for the quadratic model (Table 1). According with Yan and Hunt (1999) the overestimation of maximum cumulative germination from the bilinear model is because such estimation is established from the intercept of two linear regressions, but the

Table 1. Maximum cumulative germination obtained in experimental trials and predicted by quadratic and bilinear models. Values are means ± standard error of 5 replicates.

Cultivar	**Germination trial** (%)	**Quadratic** (%)	**Bilinear** (%)
Kancolla	72.5 ± 6.4	68.3 ± 7.1	78.3 ± 8.2
Chucapaca	90.8 ± 8.3	92.5 ± 9.4	100.0 ± 9.3
Robura	78.7 ± 8.5	80.8 ± 7.6	89.2 ± 9.6
Kamiri	80.4 ± 8.6	78.3 ± 6.4	88.3 ± 8.5
Sajama	90.0 ± 9.5	90.0 ± 8.3	100.0 ± 11.0
Amilda	85.7 ± 8.1	86.7 ± 9.2	93.3 ± 8.2
Ratuqui	80.4 ± 6.9	80.0 ± 8.0	89.2 ± 9.3
CICA	90.2 ± 10.0	89.2 ± 8.9	97.5 ± 8.7
Sayaña	85.1 ± 8.8	80.0 ± 8.9	87.5 ± 9.0
Samaranti	87.2 ± 9.2	87.5 ± 8.8	99.2 ± 9.9

Table 2. Temperature adaptability range (TAR) for 10 quinoa cultivars derived from quadrat-ic, cubic and bilinear models. Values are means ± standard error of 5 replicates.

Cultivar	Quadratic (ºC)	Cubic (ºC)	Bilinear (ºC)
Kancolla	47.0 ± 4.2	56.3 ± 6.2	63.0 ± 5.8
Chucapaca	51.8 ± 5.2	56.8 ± 5.4	66.7 ± 7.2
Robura	55.9 ± 6.0	56.8 ± 6.1	66.4 ± 6.0
Kamiri	51.5 ± 4.9	54.8 ± 5.8	62.4 ± 6.2
Sajama	55.7 ± 5.6	54.3 ± 5.0	66.8 ± 7.1
Amilda	55.0 ± 5.0	53.3 ± 4.8	63.0 ± 6.5
Ratuqui	55.6 ± 6.2	57.2 ± 6.5	67.1 ± 5.9
CICA	52.4 ± 5.5	52.0 ± 6.4	65.7 ± 6.4
Sayaña	55.8 ± 6.2	56.4 ± 5.8	63.7 ± 5.7
Samaranti	56.6 ± 5.8	58.7 ± 6.5	65.5 ± 6.8

real response of germination to temperature is curvilinear and usually smoother. In addition, Hardegree (2006) showed that near the optimum temperature there is a distinct plateau (piece-wise regression model) instead of the sharp peak that arises from the confluence of two straight lines. Nine of ten quinoa cultivars show values of temperature adaptability range (TAR) higher than 50 ºC (Table 2), which allow assuming that quinoa seeds may have a great potential to successfully germinate under field conditions, and thus will have higher probability of success than most common cereals in extremely harsh climatic conditions.

As a consequence of the thermal metabolic dynamics (Essemine *et al.,* 2010) seed germination rapidly increases between the base (minimum) temperature and optimum temperature (suboptimal range), but at temperatures higher than the optimum temperature (supraoptimal range), the germination decreases sharply. According to Sigstad & Prado (1999) water uptake by Robura and Sajama varieties at 25 ºC was very strong in the first 15 minutes and a thermal power (= heat production rate) decreased during the first 90 minutes of the germination process. After 105 to 120 minutes, the heat production showed an increase which was more evident between 310 and 390 minutes of imbibition, when radicule protrudes. From a thermal point of view, the imbibitions and heat production may be affected by low temperature. Obviously this hypothesis may be tested in lab, but in our experiment the germination at low temperature (8ºC) only 5 varieties out of 10 achieved a radicule protrusion in the first 12 hs. On the contrary, high temperatures affect biochemical reactions of seed germination metabolism. In most species from temperate regions, when the temperature increases beyond the optimum, the rate of vital processes decreases and finally ceases at a temperature of ≥ 60 °C (K'opondo *et al.,* 2011). Thermal denaturation of proteins, membrane dysfunction, oxidative stress, and decrease of metabolic efficiency have been proposed as factors that produce the decline of germination under supraoptimal temperatures (Hasanuzzaman *et al.,* 2013). According to Pineda Mejia (1999) the stress induced by high temperatures increases the percentage of abnormal germinated seeds and dead seeds in many crop species. In agreement with this finding the highest percentages of aborted and non-germinated seeds were observed under supraoptimal temperatures in all quinoa cultivars (Fig. 3). Since, the occurrence of aborted and non-germinated seeds showed great variability among quinoa cultivars. Probably it can be better explained by an exponential function instead of a linear temperature-response. In this case we can assume that the interactive effect of metabolic and genetic factors instead of the thermal factor *«per se»* is the main responsible of the occurrence of abnormalities during the quinoa germination. However the mechanism underlying abnormal germination occurring in quinoa seeds at the supraoptimal tem-

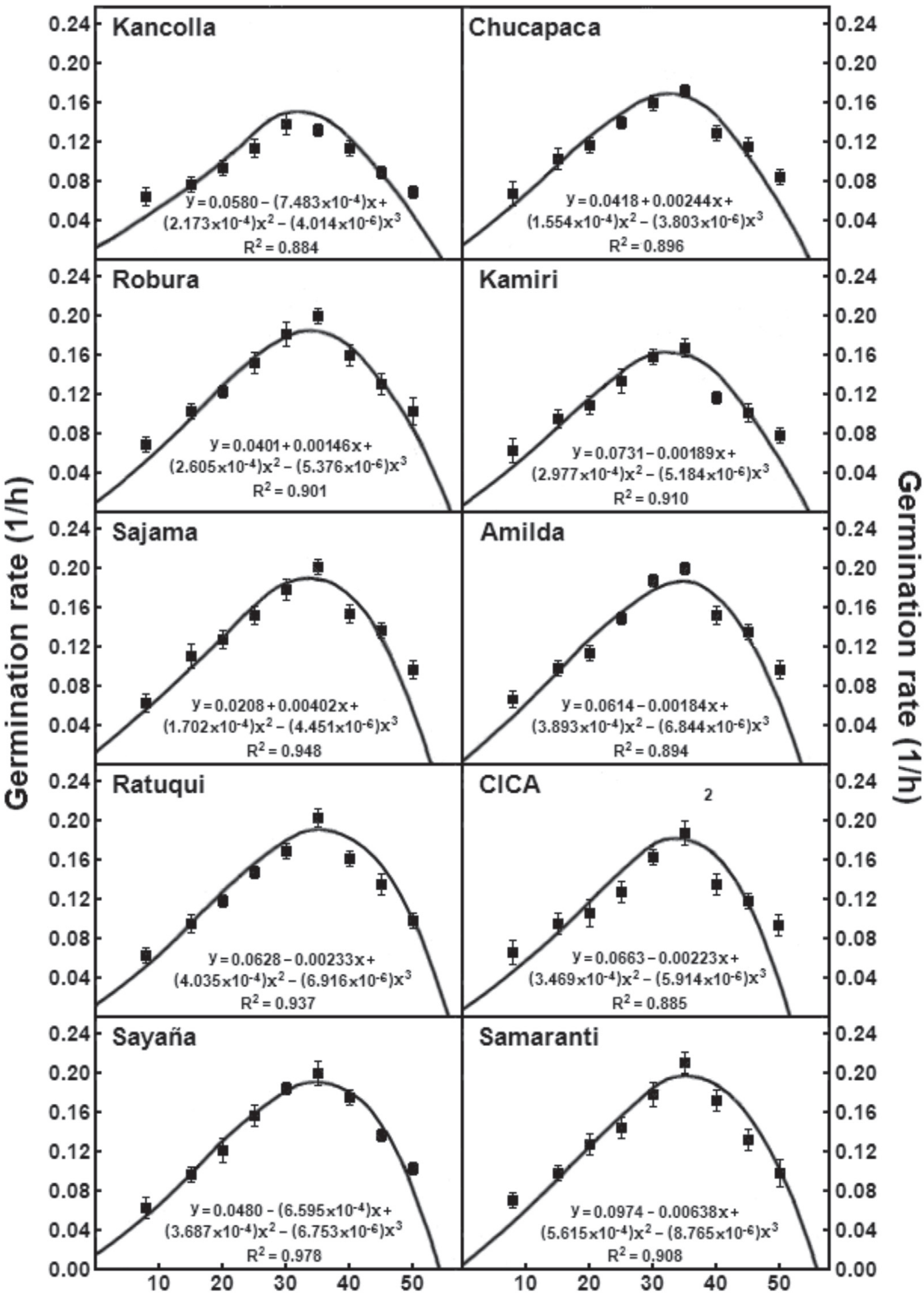

Fig. 5. Germination rate of ten quinoa cultivars incubated at different temperatures with the fitted third-order equations.Points are the observed data, solid lines are cubic models. Error bars represent one standard error of mean.

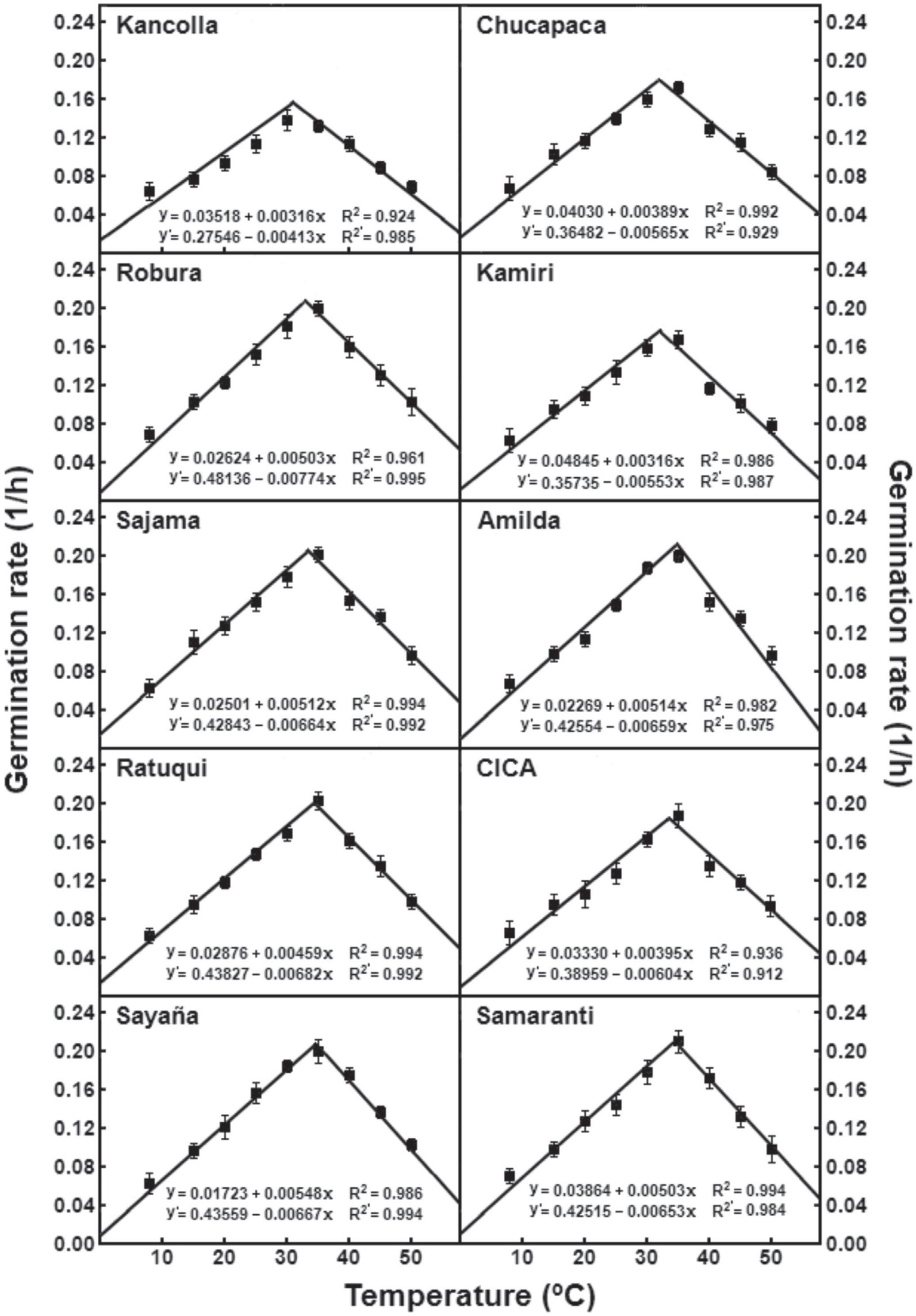

Fig. 6. Germination rate of ten quinoa cultivars incubated at different temperatures with the fitted linear equations. Points are the observed data, solid lines are bilinear models. Error bars represent one standard error of mean.

perature gradient needs further investigation. The higher percentage of aborted and non-germinated seeds observed at supraoptimal temperatures could also explain the ancestral cropping practice applied by the Andean farmers, based on the need to sow more than 100 quinoa seeds per hole to get a maximum of 10-15 plants. Supporting this assumption, both strong rises of the diurnal temperature and wide daily thermal amplitudes as consequence of the intense solar radiation and low atmospheric water content, are recorded frequently in highland arid regions of Bolivia, Peru, Chile and Argentina, where the quinoa cultivation is an ancestral practice (National Research Council, 1989). Caution is required when analysing relationships between daily thermal amplitude and quinoa germination in the field.

Cardinal temperatures i.e. T_{min}, T_{opt} and T_{max}, are important parameters to evaluate the success of seed germination and seedling establishment (Saeidnejad *et al*., 2012), and then its determination becomes important when a new crop will be introduced in a given region. Regarding to quinoa, Bois *et al.* (2006) in a study of ten quinoa cultivars from the Andean region (Bolivian Altiplano) found a linear positive correlation between germination and temperature in a temperature gradient between 2 ºC and 20 ºC. Values of T_{min} reported in this work ranged between –1.97 ºC and 0.24 ºC that were closely similar to reported here, derived from both cubic (–2.2 ºC to 0 ºC) and bilinear (–2.0 ºC to –0.5 ºC) models, respectively (Table 3). A value close to 0 ºC has been recently communicated for the T_{min} of *Chenopodium pallidicaule*, another *Chenopodiaceae* species native from the Andean region (Rodriguez *et al.,* 2016). However, values of T_{min} derived from the quadratic model were significantly higher than values reported by Bois and coworkers as well as those derived from cubic and bilinear models, indicating that these latter seem to be more reliable to establish the T_{min} of quinoa seed germination. Nonetheless, Jacobsen and Bach (1998) using a linear model reported a T_{min} value of 3 °C for a Danish quinoa cultivar. Significant dif-

Table 3. Estimates of minimum temperature (Tmin), optimum temperature (Topt) and maximum temperature (Tmax), for germination of 10 quinoa cultivars using quadratic, cubic and linear functions. Values are means ± standard error of 5 replicates.

Cultivar	Quadratic			Cubic			Bilinear		
	T_{min} (ºC)	T_{opt} (ºC)	T_{max} (ºC)	T_{min} (ºC)	T_{opt} (ºC)	T_{max} (ºC)	T_{min} (ºC)	T_{opt} (ºC)	T_{max} (ºC)
Kancolla	5.8 ± 0.4	29.8 ± 1.8	52.9 ± 4.3	–1.8 ± (–0.2)	32.0 ± 3.6	54.5 ± 4.1	–1.8 ± (–0.2)	32.0 ± 2.8	61.2 ± 6.7
Chucapaca	4.2 ± 0.3	30.0 ± 3.1	56.0 ± 5.0	–2.0 ± (–0.3)	33.0 ± 2.9	54.8 ± 5.5	–2.0 ± (–0.2)	32.0 ± 3.4	64.7 ± 6.7
Robura	4.3 ± 0.4	32.0 ± 2.0	60.2 ± 6.3	–0.8 ± (–0.1)	33.0 ± 3.3	56.0 ± 4.9	–0.7 ± (–0.1)	32.7 ± 3.4	65.7 ± 5.9
Kamiri	4.5 ± 0.3	30.5 ± 3.2	56.0 ± 6.0	–0.3 ± (–0.0)	32.5 ± 3.6	54.5 ± 6.1	–1.2 ± (–0.3)	31.8 ± 2.8	61.2 ± 4.7
Sajama	4.5 ± 0.4	32.2 ± 3.6	60.2 ± 5.9	–1.5 ± (–0.2)	33.2 ± 2.5	52.8 ± 3.9	–2.0 ± (–0.2)	33.2 ± 3.3	64.8 ± 6.8
Amilda	4.8 ± 0.5	32.0 ± 3.0	59.8 ± 6.3	0.0 ± 0.0	35.0 ± 4.1	53.3 ± 5.0	–1.2 ± (–0.1)	34.7 ± 3.3	61.8 ± 5.3
Ratuqui	5.2 ± 0.5	32.5 ± 2.2	60.8 ± 6.0	–1.5 ± (–0.2)	35.2 ± 3.0	55.7 ± 5.0	–1.6 ± (–0.2)	34.5 ± 4.1	65.5 ± 6.3
CICA	5.8 ± 0.6	32.0 ± 3.7	58.2 ± 4.9	–0.5 ± (–0.1)	33.8 ± 2.9	51.5 ± 4.8	–0.5 ± (–0.1)	33.5 ± 3.2	65.2 ± 5.9
Sayaña	5.2 ± 0.5	33.2 ± 2.9	61.0 ± 6.4	–2.2 ± (–0.3)	35.0 ± 3.1	54.2 ± 5.7	–0.2 ± (–0.0)	34.6 ± 3.0	63.5 ± 6.8
Samaranti	4.2 ± 0.5	32.5 ± 3.4	60.8 ± 5.2	–0.5 ± (–0.1)	36.0 ± 4.0	56.0 ± 5.7	–1.0 ± (–0.2)	34.0 ± 3.0	64.5 ± 6.1

ferences between field and controlled conditions in T_{min} values were also reported for quinoa cultivars (Bertero *et al.,* 1999, cited by Bois *et al.,* 2006). Disparity in T_{min} values within the same species occurring under both field and controlled conditions appears as a common trait in most plants, but it cannot be explained by a simple factor such as seed size or seed water status (Wang *et al*., 2004; Naim and Ahmed, 2015). Complex maternal effects and the variation in individual seed sensitivity to temperature are interacting and trigger the disparity of germination response at intraspecific level (Vange *et al*., 2004).

The T_{opt} values calculated from linear and polynomial regression models showed variation among cultivars but in all models the maximum germination occurred at T_{opt} values from 29.8 ºC to 36 °C, being slightly lower in the quadratic model. In agreement with our results, Jacobsen and Bach (1998) reported a value of T_{opt} between 30 ºC and 35 ºC for maximum germination of different populations of a Danish quinoa cultivar. By contrast maximum temperature (T_{max}) was higher in the bilinear model compared with both quadratic and cubic models. According with Hardegree (2006) when the regression line is forced to intercept the x-axis at supraoptimal temperatures, an overestimation of the T_{max} occurs. By comparison of data, bilinear (mean $R^2 = 0.975$) and cubic (mean $R^2 = 0.914$) models seem to have comparative advantage over the quadratic model (mean $R^2 = 0.825$) to estimate cardinal temperatures of quinoa seed cultivars.

CONCLUSIONS

Our results showed that high and low temperature affects not only the rate but also the maximal germination. On the basis of a cost/benefit analysis, data of this study indicates that to perform quinoa cultivation in mountainous regions it is preferable planning the crop on an altitudinal ecological zoning base, that is to say, select one or more cultivars for each altitudinal level. This implies to know both thermal requirements and germinative fitness (germination percentage, germination rate, cardinal temperatures, non-germinated seeds and aborted seeds) of quinoa cultivars for each selected site to choose more suitable cultivars before starting the crop.

ACKNOWLEDGEMENTS

This work was supported by the Agencia de Promoción Científica y Tecnológica (grant PICT 23153) and Fundación Miguel Lillo (Miguel Lillo 251, T4000JFE, Tucumán, Argentina).

REFERENCES

Abugoch James L. E. 2009. Quinoa (*Chenopodium quinoa* Willd.): composition, chemistry, nutritional, and functional properties. Advances in Food Nutrition Research 58: 1-31.

Bazile D., Fuentes F. F., Mujica A. 2013. Historical perspectives and domestication of quinoa, in: Bhargava, A., Srivastava S. (Eds.), Quinoa: Botany, Production and Uses. CAB International, Wallingford, UK, pp. 16-35.

Bertero H. D. 2001. Effects of photoperiod, temperature and radiation on the rate of leaf appearance in quinoa (*Chenopodium quinoa* Willd.) under field conditions. Annals of Botany 87: 495-502.

Bertero H. D., King R. W., Hall A. J. 1999. Modelling photoperiod and temperature responses of flowering in quinoa (*Chenopodium quinoa* Willd.). Field Crops Research 63: 19-34.

Berti M. T., Johnson B. L. 2008. Seed germination response of cuphea to temperature. Industrial Crops and Products 27: 17-21.

Bewley J. D., Black M. 1994. Seeds: Physiology of Development and Germination, second ed. Plenum Press, NewYork, USA.

Bois J. F., Winkel T., Lhomme J. P., Raffaillac J. P., Rocheteau A. 2006. Response of some Andean cultivars of quinoa (*Chenopodium quinoa* Willd.) to temperature: effects on germination, phenology, growth and freezing. European Journal of Agronomy 25: 299-308.

Bonhomme R. 2000. Bases and limits to using ‘degree day’ units. European Journal of Agronomy 13: 1-10.

Bove J., Jullien M., Grappin P. 2001. Functional genomics in the study of seed germination. Genome Biology, 3 (1): reviews 1002.1-1002.5.

Choukr-Allah R., Rao N. K., Hirich A., Shahid M., Alshankiti A., Toderich K., Gill S., Butt K. U. R. 2016. Quinoa for marginal environments: toward future food and nutritional security in MENA and central Asia regions. Frontiers in Plant Science 7: 346.

Covell S., Ellis R. H., Roberts E. H., Summerfield R. J. 1986. The influence of temperature on seed germination rate in grain legumes. Journal of Experimental Botany 37: 705-715.

Dixon M., Webb E. C. 1979. Enzymes. Prentice Hall Press, New Jersey, USA.

Dumur D., Pilbeam C. J., Craigon J. 1990. Use of the Weibul function to calculate cardinal temperatures in Faba bean. Journal of Experimental Botany 41: 1423-1430.

Essemine J., Ammar S., Bouzid S. 2010. Impact of heat stress on germination and growth in higher plants: physiological, biochemical and molecular repercussions on mechanisms defence. Journal of Biological Sciences 10: 565-572.

González J. A., Prado F. E. 1992. Germination in relation to salinity and temperature in *Chenopodium quinoa* (Willd.). Agrochimica 36: 101-108.

Gonzalez J. A., Konishi Y., Bruno M., Valoy M., Prado F. E. 2011. Interrelationships among seed yield, total protein and amino acid composition of ten quinoa (*Chenopodium quinoa*) cultivars from two different agroecological regions. Journal of the Science of Food and Agriculture 92: 1222-1229.

González J. A., Eisa S., Hussin S., Prado F. E. 2015. Quinoa: an Incan Crop to Face Global Changes in Agriculture. In: Murphy, K.S., Matanguihan, J. (Eds.). Quinoa: Improvement and Sustainable Production. Wiley-Blackwell, Hoboken, NJ, USA, pp. 1-18.

Hardegree S. P. 2006. Predicting germination response to temperature. I. Cardinal-temperature models and subpopulation-specific regression. Annals of Botany 97: 1115-1125.

Hasanuzzaman M., Vahar K., Fujita M. 2013. Extreme temperature responses, oxidative stress and antioxident defense in plants, in: Vahdati, K., Leslie, C. (Eds.), Abiotic Stress – Plant Responses and Applications in Agriculture. InTech, Croatia, pp. 169-203.

Jacobsen S. E., Bach A. P. 1998. The influence of temperature on seed germination rate in quinoa (*Chenopodium quinoa* Willd). Seed Science and Technology 26: 515-523.

Jacobsen S. E., Monteros C., Christiansen J. L., Bravo L. A., Corchera L. J., Mujica A. 2005. Plant responses of quinoa (*Chenopodium quinoa* Willd.) to frost at various phenological stages. European Journal Agronomy 22: 131-139.

KakaniV. G.,Prasad P. V. V.,Craufurd P. Q., Wheeler T. R. 2002. Response on in vitro pollen germination and pollen tube growth of groundnut (*Arachis hypogaea* L.) genotypes to temperature. Plant, Cell and Environment 25: 1651-1661.

Kamkar B., Al-Alahmadi M. J., Mahdavi-Damghani A., Villalobos F. J. 2012. Quantification of the cardinal temperatures and thermal time requirement of opium poppy (*Papaver somniferum* L.) seeds to germinate using non-linear regression models. Industrial Crops and Products 35: 192-198.

K'Opondo F. B. O., Groot S. P. C., Van Rheenen H. A. 2011. Determination of temperature and light optima for seed germination and seedling development of spiderplant (*Cleome gynandra* L.) morphotypes from western Kenya. Annals Biological Research 2: 60-75.

Luna B., Pérez B., Torres I., Moreno J. M. 2012. Effects of incubation temperature on seed germination of Mediterranean plants with different geographical distribution ranges. Folia Geobotanica 47: 17-27.

Naim A. H., Ahmed F. E. G. 2015. Variation in thermal time model parameters between two contrasting chickpea (*Cicer arietinum*) cultivars. Agricultural Sciences 6: 1421-1427.

National Research Council. 1989. Lost Crops of the Incas: Little-Known Plants of the Andes with Promise for Worldwide Cultivation. National Academy Press, Washington, DC, USA.

Nonogaki H., Bassel G. W., Bewley J. D. 2010. Germination still a mystery. Plant Science 179: 574-581.

Pineda Mejia R. 1999. Effects of stress temperatures of germination on polyamine titers of soybean seeds. Ph.D. thesis, Iowa State University, USA, pp. 97.

Prado F. E., Boero C., Gallardo M., González J. A. 2000. Effect of NaCl on germination, growth, and soluble sugar content in *Chenopodium quinoa* Willd. seeds. Botanical Bulletin of *Academia Sinica* 41: 27-34.

Prado F. E, Fernández-Turiel J. L., Tsarouchi M., Psaras G. K., González J. A. 2014. Variation of seed mineral concentrations in seven quinoa cultivars grown in two agroecological sites» *Cereal Chemistry* 91: 453-459.

Risi J., Galwey N. W. 1984. The Chenopodium grains of the Andes: Inca crops for modern agriculture. Advances in Applied Biology 10: 145-216.

Rodriguez J. P., Jacobsen S. E., Sørensen M., Andreasen C. 2016. Germination responses of cañahua (*Chenopodium pallidicaule* Aellen) to temperature and sowing depth: a crop growing under extreme conditions. Journal of Agronomy and Crop Science. DOI: 10.1111/jac.12158.

Rosa M., Hilal M., González J. A., Prado F. E. 2004. Changes in soluble carbohydrates and related enzymes induced by low temperature during early developmental stages of quinoa (*Chenopodium quinoa* Willd.) seedlings. Journal of Plant Physiology 161: 683-689

Saeidnejad A. H., Kafi M., Pessarakli M. 2012. Evaluation of cardinal temperatures and germination responses of four ecotypes of *Bunium persicum* under different thermal conditions. International Journal of Agriculture and Crop Sciences 4: 1266-1271.

SAS Statistical Analysis Software for Windows. Release 9.1.3., 2010. Cary, North Carolina, USA.

Seepaul R., Macoon B., Reddy K. R., Baldwin B. 2011. Switchgrass (*Panicum virgatum* L.) intraspecific variation and thermotolerance classification using in vitro seed germination assay. American Journal of Plant Sciences 2: 134-147.

Strenske A., Soares de Vasconcelos, E., Egewarth V. A, Michelon Herzog N. F., de Matos Malavasi M. 2017. Responses of quinoa (*Chenopodium quinoa* Willd.) seeds stored under different germination temperaturas. Acta Scientiarum. Agronomy Maringá 39: 83-88

Vange V., Heuch I., Vandvik V. 2004. Do seed mass and family affect germination and juvenile performance in *Knautia arvensis*? A study using failure-time methods. Acta Oecologica 25: 169-178.

Vega-Gálvez A., Miranda M., Vergara J., Uribe E., Puente L., Martínez E. A. 2010. Nutrition facts and functional potential of quinoa (*Chenopodium quinoa* willd.), an ancient Andean grain: a review. Journal of the Science of Food and Agriculture 90: 2541-2547.

Wang R., Bai Y., Tanino K. 2004. Effect of size and sub-zero imbibition-temperature on the thermal time model of winterfat (*Eurotia lanata* (Pursh) Moq.). Environmental and Experimental Botany 51: 183-197.

White S. N., Boyd N. S., Van Acker R. C. 2015. Temperature thresholds and growing-degree-day models for red sorrel (*Rumex acetosella*) ramet sprouting, emergence, and flowering in wild blueberry. Weed Science 63: 254-263.

Yan W., Hunt L. A. 1999. An equation for modeling the temperature response of plants using only the cardinal temperature. Annals of Botany 84: 607-614.