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Evaluation of cardinal temperatures and germination responses of four ecotypes of *Bunium persicum* under different thermal conditions

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ABSTRACT: Considering the importance of germination on plants' domestication processes, cardinal temperatures' determinations appear to be a major step. Black cumin (Bunium persicum) is an advantageous medicinal plant which is forced into the endangered plants group because of inappropriate management practices and the lack of any published information about the cardinal temperatures effects on this species germination. In order to investigate germination response of of Black cumin to different temperatures, an experiment was conducted with four ecotypes of Bunium persicum (including, CE_2, CE_4, CE_8, and CE_9 ecotypes) from different parts of Iran at nine levels of temperature (4, 8, 12, 16, 18, 20, 24, 28, and 32 ℃**).** Three models were fitted to determine the germination responses to temperature and comparing the efficiency of each model. Results indicated different correlations among the observed and simulated germination rate values for all ecotypes. Calculated values by the Intersected-line model (ISL) revealed the values from 0.35 to 3.33 ℃ for the base, 16.19 to 22.14 ℃ for the optimal, and 29.74 to 31.52 ℃ for the maximum temperatures. Fitted Quadratic Polynomial Model (QPN) showed significant correlation between the observed and simulated values for CE_8 and CE_9 ecotypes, but not for CE_2 and CE_4. According to the results of the five parameters of the beta model, cardinal temperatures for the evaluated ecotypes are similar to the calculated values with Quadratic Polynomial Model to some extent. Generally, ecotypes from the elevated habitats showed lower base, optimal, and maximum temperatures.

Keywords: Bunium persicum, Ecotypes, Germination, Temperature

INTRODUCTION

Black cumin (Bunium persicum) is a medicinal plant from the Apiaceae family, grown specifically in different regions of Iran. B. persicum seeds are called "zireh kuhi", meaning "wild cumin", Black cumin, Zira Kermani, Persian Zira, and Kaka Zira are used as a culinary spice (Mortazavi et al., 2010). In the indigenous system of medicines, seeds are regarded as stimulants and carminatives and found to be useful in diarhea and dyspepsia (Abduganiew et al., 1997). Also, this plant is used for culinary purposes and for flavouring foods and beverages. It should be noted that the name of black cumin is sometimes given to entirely unrelated spice Nigella sativa L (Pourmortazavi et al., 2005). It must be noted that alarmingly the plant has been forced into the endangered category due to the recent relentless extraction of seeds obtained from wild habitats. The main depletion factor and cause of endangerment of this species has been found to be the thoughtless, improper, and unscientific commercial collection of its seeds for rapid financial gains. The competition for its seeds is so severe that, instead of collecting the ripe seed, the entire plant is removed even when the seeds are immature (Azizi et al., 2009). If this improper and irresponsible trend of harvesting the seeds of this plant is continued, regretfully, it would lead to the extinction of this endangered species.

Seed germination is known as a key process for plants regeneration and it gained doubled importance for endangered plants which are vital for protecting diversity and the composition of plants communities (Fenner and

Thompson, 2005). Providing the basic information about the factors which affect germination process leads to a better understanding of plant life cycle and growth and facilitate the conservation and management of these species in modern agricultural systems (Van der Valk and Pederson, 1989).

Temperature is one of the most critical factors affecting the seed germination (Verma et al., 2010), probably the most important factor (Nerson, 2007). Onset, potential, and rate of germination have been significantly affected by temperature (Flores and Briones, 2001).

Optimal temperatures determination is also necessary in case that the suitability of a new region needs to be evaluated for introducing and cultivating a new plant (Adam et al., 2007).

Flores and Briones (2001) evaluated germination responses of six desert species to different temperatures and reported that along with increasing temperature, germination initiated earlier and the mean germination time (MGT) was decreased. In another study, Jami Al-Ahmadi and Kafi (2007) studied the optimal temperatures for germination of Kochia scoparia and revealed that kochia is able to adjust its germination over a wide range of temperatures, from 3.5 °C (T_b) to 50 °C (T_{max}), with an optimum germination temperature of 24 °C. Investigation of the thermal time requirement for germination of three turfgrass species showed that the final germination percentage was little affected by temperature, indicating that the base temperature for germination (T_b) is relatively constant within the seed populations. This turfgrass temperature determination experiment also suggested that poor establishment of Kentucky bluegrass (Poa pratensis L.) may partly be due to a larger thermal time required for germination (Larsen and Bibby, 2005). Qiu et al. (2006) used thermal time models to predict seedling emergence of orchardgrass (Dactylis glomerata L.) under alternating temperature regimes. In another study, Khosh-Khui and Bonyanpour (2006) found a temperature range between 15 to 25 °C to be the optimum temperature for cumin (Cuminum cyminum) seed germination, and the maximum germination percentage was achieved at 15 °C.

Optimal, upper and lower critical temperatures for germination of many agronomic plants have been well established (Jensen, 2001). However, the medicinal and endangered plants received less attention and no data are available on the optimal and critical temperatures for germination of Bunium persicum. Considering this fact, the objectives of the present investigation were to evaluate the effects of different temperatures on germination properties of Bunium persicum and to determine the cardinal temperature of germination for this valuable and endangered species.

MATERIALS AND METHODS

Plant materials and samples collection

The experiment was conducted at Physiological Research Laboratory, Ferdowsi University of Mashhad, Iran, during the year 2010. With respect to the differences among the ecotypes of B. persicum and with the aim of finding the variations, the main habitats of the plant were located and climatic properties of each region were evaluated. Afterward, four ecotypes were collected from the natural habitats of plant located on 4 provinces of Iran, including Kerman (Joopar, Margiri mountain), Qazvin (Alamoot mountain), Khorasan Razavi (Kelat, Khajeh Forest), and Fars (Estahban, Toodaj mountain) provinces. Soil samples of each area were also collected and analyzed. Climatic information and soil analysis data of each region are presented in Table 1. Seeds were hand threshed to minimize seed damage. They were also separated manually from other particles with similar size and empty or off-size seeds. All ecotypes seeds were stored at 4 ^{o}C for 60 days in order to provide cold requirement of the seeds to onset germination, based on the experience of the authors (unpublished).

Germination test

Seed germination of the evaluated ecotypes was assessed using four replicates of 25 seeds. Seeds of each replicate were placed on Petri dishes. Filter papers were soaked in distilled water, in advance and water was added to the Petri dishes during the test if needed. In order to evaluate the response of germination to different temperatures and determining base, optimum, and maximum temperatures for germination process, 8 treatments were applied, including 4, 8, 12, 16, 20, 24, 28, and 32 ^{o}C under dark conditions and 50% relative humidity in growth chambers. Germination was recorded every day for 15 days with the appearance of a radicle of 2mm. Germination rate was calculated as mean germination time (MGT) using the following formula:

$MGT = \Sigma(fx) / \Sigma x$

Where, “f” is the days from the beginning of the experiment and “x” is the number of the newly germinated seeds on that day (Khajeh-Hosseini et al., 2010).

Statistical analysis and model evaluation

A normality test was performed for all data and data transformation was performed when required (arcsin √%). Cardinal temperatures were determined using three regression models between inverse of time span to 50% germination and various temperatures.

Intersected-lines model

Intersected lines models were fitted to the data of the reciprocals of time to 50% of germination at various temperatures. The following equations were used:

$$f = if(T < T\_o, region\ 1\ (T), region\ 2\ (T)) \quad (1)$$

$$Region\ 1(T) = b\ (T - T\_b) \quad (2)$$

$$Region\ 2(T) = c\ (T_m - T) \quad (3)$$

Where, T is temperature treatment, T_b, T_{opt}, and T_{max} are base, optimum, and maximum temperatures, respectively.

Five-parameters Beta model

Five-parameters Beta model is a model for relationship across development rate and temperatures. The Beta function is widely utilized to provide a flexible family of nonsymmetrical, unimodel probability density functions with fixed end points which set aside points of inflexion on other side of the model (Yin et al., 1995). Beta function was defined as below:

$$\text{Development rate} = \exp(\mu)\ 〚(T - T\_b)〛 \hat{}a\ 〚(T\_c - T)〛 \hat{}\beta \quad (4)$$

$$T\_o = (\alpha T\_m + \beta T\_b)/(\alpha + \beta) \quad (5)$$

Where, T, T_b, T_c, T_o, and T_m are treatment, base, ceiling, optimum, and maximum temperatures, respectively. μ, α, and β are model parameters.

Quadratic polynomial model

A quadratic polynomial equation was also used to cardinal temperatures determination as follows:

$$f = a + bT + cT^2 \quad (6)$$

$$T_o = b + 2cT \quad (7)$$

Where, T is temperature treatment and a, b, and c are model parameters.

RESULTS AND DISCUSSION

Germination modeling

Results of the three fitted models for germination responses of the evaluated ecotypes revealed different correlations between the observed and simulated germination rate values of Black cumin.

According to the results of the Intersected-line model (ISL), cardinal temperatures were different across the ecotypes (Fig. 1). Base temperature (T_b) was calculated for the CE_2, CE_4, CE_8, and CE_9 ecotypes as 3.33, 4.14, 0.27, and 0.35 ℃, respectively. Optimal temperature was varied from 16.19 to 22.14 ℃ among the ecotypes. The ecotypes also showed different maximum temperatures from 29.74 to 31.52 ℃.

Fitted Quadratic Polynomial Model (QPN) showed significant regression between the observed and simulated values in some ecotypes, but not all of them (Fig. 2). For the CE_2 and CE_4 ecotypes, simulated results showed less regression with the observed values (Figs. A.2 and B.2). But, the CE_8 and CE_9 ecotypes showed higher regression than the observed values (Figs. C.2 and D.2). The values of 1.70 and 1.85 for the base, 16.33 and 16.63 for the optimum, and 31.57 and 30.81 ℃ for the maximum temperatures were calculated for the CE_8 and CE_9 ecotypes, respectively. It seems that this model is not appropriate for the CE_2 and CE_4, but a good one for the CE_8 and CE_9 ecotypes.

Results of five parameters beta model indicated that the cardinal temperature for the evaluated ecotypes is somehow similar to the calculated values with Quadratic Polynomial Model (Fig. 3). Simulated values for T_b are 3.23, 4.00, 0.22, and 1.85 for the CE_2, CE_4, CE_8 and CE_9 ecotypes, respectively. Considering the results of the optimum and the maximum temperatures revealed significant correlation between the observed and the simulated values.

Variation between the calculated values is seemed to be related to the differences among the ecotypes which originated from the regions with diverse climatic conditions. As it was shown on Fig. 1, the CE_4 ecotype is related to Kelat region with 920 m altitude. Since this area has warmer climate compared with the other origins of the evaluated ecotypes, the seeds are adapted with this condition and showed a higher T_b. The optimal and the maximum temperatures of this ecotype were also higher than that of the others, because of the explained reason.

The CE_2 and CE_8 ecotypes are originated from the areas where the climatic conditions are very similar. Thus, the similarities between the cardinal temperatures, including base, optimum, and maximum are reasonable. The considerable point about the simulated cardinal temperatures of the CE_9 ecotype is with respect to the fact that the climatic properties of the origin of this ecotype are somewhat different (the altitude of Margiri mountain is 2950 m) than that of the other ecotypes. Therefore, it was expected that this ecotype would have lower base and maximum temperatures. But, the difference between this ecotype and the CE_8 ecotype was not significant. The CE_8 ecotype showed even lower base and maximum temperatures.

Based on this result, it seems that Bunium persicum has the ability to adjust itself to the environmental conditions to some extent, especially to the temperature. But, after some point, this process is not continued and plant encounters with the problems of the environmental conditions.

Cardinal temperatures for germination have been studied by many researchers for various groups of crops and some medicinal plants. For instance, cardinal temperature for canola as a winter crop was investigated and they reported 4.2 to 31 ℃ as the range of cardinal temperature (15). The cardinal temperatures for germination and early growth of different accessions of two Lesquerella species indicated that the collected accessions from the elevations above 2000m performed better at warmer temperatures, whereas those collected from the elevations below 2000m tended to perform better at cooler temperatures (2). It seems that this difference is mostly related to the genetic differences between these two species.

Overall and according to the correlation values, it can be concluded that for the CE_2 and CE_4 ecotypes, intersected-lines model appeared to be the most appropriate model to calculate the cardinal temperatures. But, for the CE_8 and CE_9 ecotypes, Five-parameter Beta model was a better fit compared with the other models. Quadratic polynomial model values did not revealed satisfactory correlation between the observed and the simulated values.

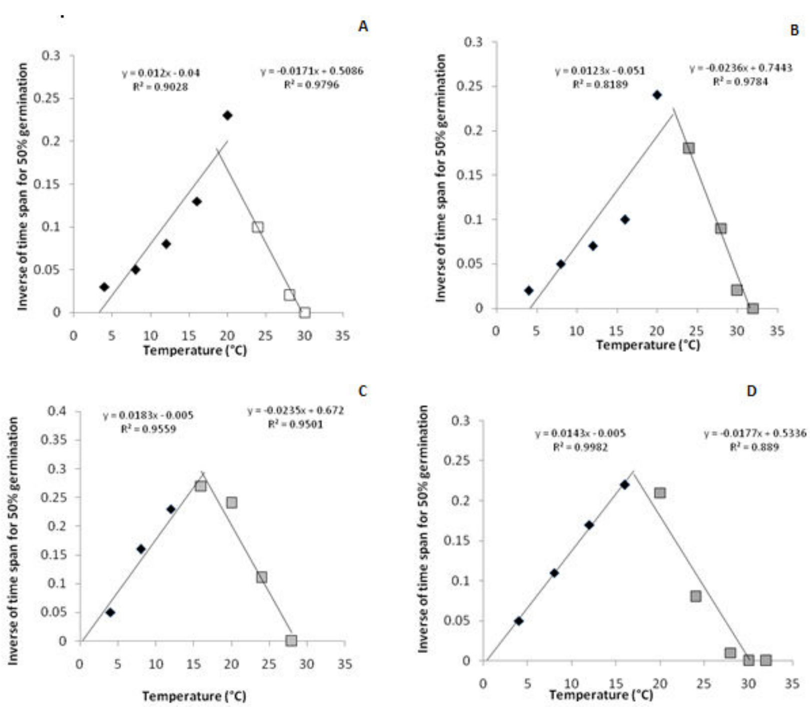

Figure 1. Germination rate index (inverse time taken to achieve 50% germination) of Bunium persicum, including CE2 (A), CE4 (B), CE8 (C) and CE9 (D) ecotypes in response to temperature using Intersected-lines model.

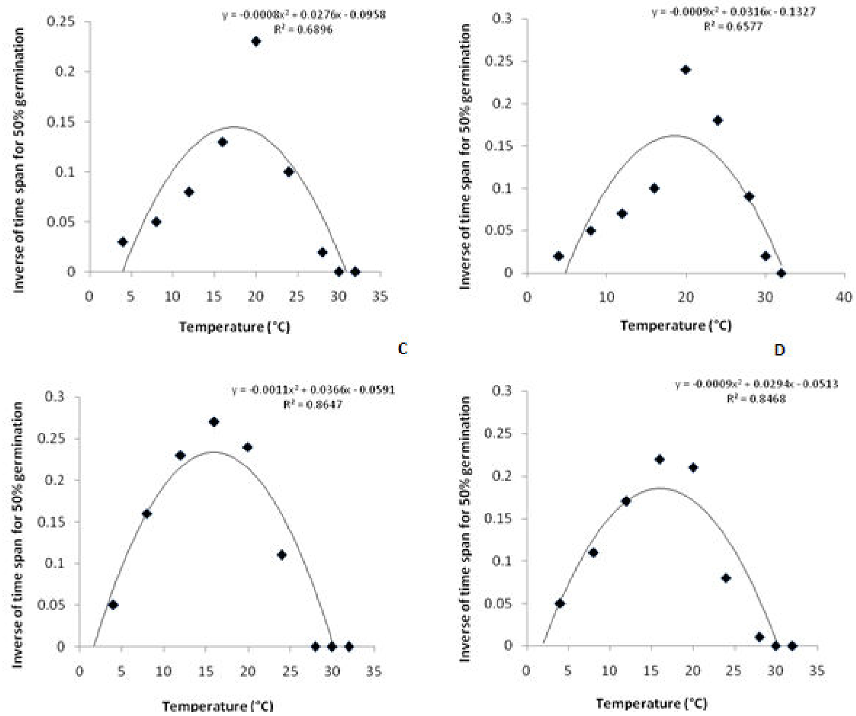

Figure 2. Quadratic polynomial model of the germination rate index (inverse time taken to achieve 50% germination) of Bunium persicum including CE2 (A), CE4 (B), CE8 (C), and CE9 (D) ecotypes in response to temperature.

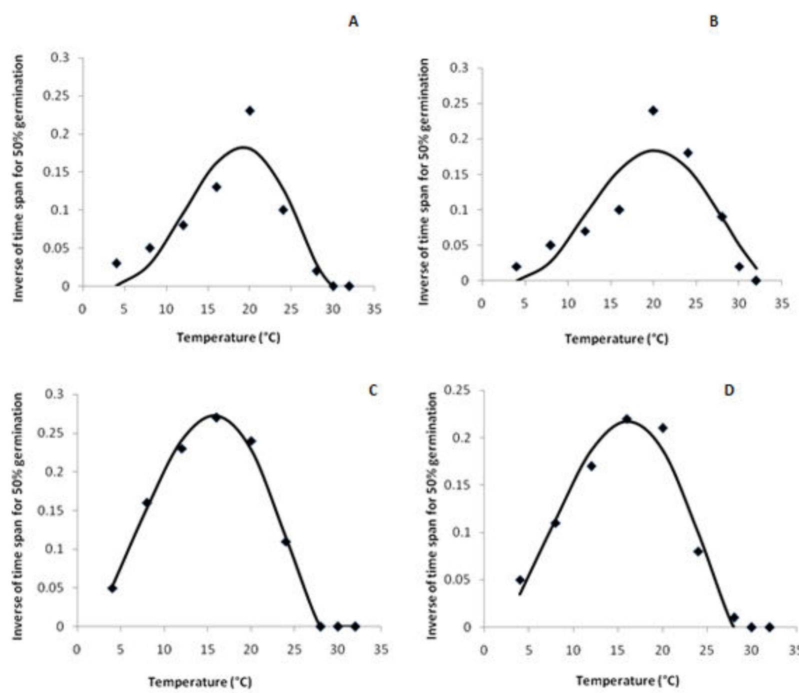

Figure 3. Fitted Five-parameters Beta model for germination rate index (inverse time taken to achieve 50% germination) of Bunium persicum, including CE2 (A), CE4 (B), CE8 (C), and CE9 (D) ecotypes in response to temperature.

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