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Research and Full Length Article:

Investigating the Germination Characteristics of *Poterium sanguisorba* Seeds under the Influence of Thermal Treatments for Pasture Establishment

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Abstract. Common burnet (*Poterium sanguisorba*) is a perennial herb from rose family (*Rosaceae*) which is used to construct pastures. The studied plant can resist against freezing, cool and drought. The present study was conducted to study the seed germination parameters of common burnet in different thermal treatments as completely randomized design in 4 replications in 2013. Treatments involved the effects of constant temperature including 5, 10, 15, 20, 30, 35 and 45°C on germination parameters of 25 seeds which were used at each replication. The results showed that the effects of different thermal treatments on seed germination of common burnet were significant so that the lowest germination speeds at 5 and 10°C were 0 and 2.78 seeds a day and the highest germination speeds at 35 and 30°C were 8.95 and 7.77 seeds a day, respectively. The longest plume lengths were observed at temperatures of 10 (4.94 cm) and 15 °C (4.47 cm) and the shortest plume lengths were 35 and 45° (1.28 and 0 cm, respectively). The longest radicle lengths also were observed at 10 (5.13 cm) and 15 °C (5.05 cm) and the shortest ones occurred at 35 and 45 °C (0.88 and 0 cm, respectively). According to the fitted regression models between germination speed and temperature, minimum, optimum and maximum values of temperature were obtained at the ranges of 3.38-6.65, 26.82-34.5 and 45-46.78°C, respectively. According to the seed germination of studied plant at maximum and minimum temperatures, it can be used in constructing the pastures in arid and semi-arid areas.

Key words: Temperature, *Poterium sanguisorba*, Germination, Pasture establishment

Introduction

The first steps to conserve a plant species and improve biodiversity in rangelands are to identify the species and study the germination parameters, vegetation and reproduction. Common burnet -*Poterium sanguisorba*- (Rosaceae family) is a perennial herb with the erected stems (Yin, 1996). The root system can penetrate into the soil depth of 70 to 100 cm. The best growth of plant occurs at adequate light but can tolerate the shade conditions too (Ogle, 2002). In the rain-fed conditions, the species has shown the highest potential of seed production in comparison with 11 different grass species; therefore, it is suitable to be planted in cold climates and is tolerant to freezing, cool and drought (Fisher *et al.*, 1987). Common burnet is used to construct natural and cultivated pastures (Hickman, 1993). The nutritional value of its fodder is of high importance for all livestock, especially sheep and is the same as those of alfalfa and Onobrychis sativa while producing no gas in livestock. It has high protein and carotene contents. It remains green and gastronome during its growth and development in the glacial coldness. The amount of its protein is high in spring and decreases up to late summer (Welch, 2004). The obtained results indicate the important role of the studied plant in soil conservation and enrichment. The fodder yield and seed production of common burnet together with its nutritional value have been evaluated to be higher than those of grasses (Rasoli and Shabani Tabari, 1996). The species *Sanguisorba minor* has been used potentially in the pasture improvement and grazing development up to late fall and winter due to relatively long growth season (Nelson, 2013).

Seed germination is considered as one of the most important events for the success of most plants. It is one of the most critical stages in seedling establishment (Jami Al-Ahmadi and Kafi, 2007; Parmoon *et al.*, 2015). This stage of growth is a complete physiological process which is affected by several environmental and genetic factors including temperature, light and moisture among which temperature affects the seed dormancy and germination importantly (Krichen *et al.*, 2014; Ordonez-Salanueva *et al.*, 2015; Alvarado & Bradford, 2002) starting with the appearance and elongation of plunume and radicle and the allocation of the stored nutrients to embryonic axis. The success in crop production greatly depends on the uniformity and fast establishment of seeds which correlate closely with the germination percent and amount (Eberle *et al.*, 2014).

To start the germination, seeds of plant species need a minimum temperature and the germination increases with the increased temperature. Finally, germination will stop with the further increase in temperature up to the seed tolerance ceiling which is called maximum germination temperature. Minimum, optimum and maximum temperatures are generally called cardinal (major) temperatures (Naaghd *et al.*, 2002). Determination of cardinal temperatures can be effective in evaluating the species geographical constraints and their plantation time and predicting the growth and development stages of agricultural crops (Mahmodi *et al.*, 2007). Accordingly, germination is also limited by temperature under proper moisture conditions (Kauth & Biber, 2015). Germination response to temperature depends on several factors including plant species, variety, vegetation area, seed quality and time after harvesting (Rezaei, 2002). Germination speed is one of important aspects of seed which can be considered as a limiting factor in the plant establishment (Xue *et al.*, 2012). Researchers have reported a linear relationship between temperature and germination speed in some plant species and mostly use the linear regression to describe the relationship between temperature and germination speed (Zafaranieh, 2014). Several studies investigated the effects of temperature on seed germination of different species (Kamkar *et al.*, 2012; Gibertson *et al.*, 2014; Kaldy *et al.*, 2015).

The effect of temperature on germination which can be stated as cardinal temperatures is required to develop the seed germination of predicting models in plant species (Derakhshan *et al.*, 2013). Germination percent, germination speed and seedling growth (elongation of plume and radicle) are affected by temperature meaning that the speed and germination percent decrease at temperatures lower than the optimum level of germination and increase continuously with the increased temperature up to optimum level; however, the seed is damaged following the increased temperature beyond the optimum level while approaching to lethal temperature which causes a reduction in germination percent and speed (Khaleghi and Moallemi, 2009).

Since identifying the effective factors on germination can lead to present new strategies to manage herbal plants and it is necessary in rangeland conservation and also, according to the fact that there is not enough information on the required germination conditions of *Poterium sanguisorba*, it is necessary to investigate the effects of environmental factors on germination. So, the most important purposes of this study are to determine the cardinal temperature of seed germination parameters in *Poterium sanguisorba* and recognize the relationship between temperature and these parameters to construct a pasture. The results of this study will be useful for characterizing the eco-physiology of seed germination of *Poterium sanguisorba*. Such knowledge may be useful for identifying the best planting dates for this plant in the range climates and regions.

Materials and Methods

In order to study the effects of different temperatures on seed germination of *Poterium sanguisorba*, an experiment was conducted on the basis of completely randomized design with four replications in the laboratory of Soil Science Department of Agricultural Faculty of Ferdoosi University, Mashhad, Iran in 2013. Seeds of *Poterium sanguisorba* were collected from rangelands and kept at ambient temperature till the beginning of experiment. Different temperatures consisted of 5, 10, 15, 20, 30, 35 and 45°C and photoperiod involved 16 hours of illumination (light intensity of 500 LUX) and 8 hours of darkness. Then, 25 seeds were arranged in four repetitions in the sterilized Petri dishes with the mouth diameter of 9 cm containing Wattmen filter papers. Daily counting of germinated seeds (with consideration of radicle length of 2 mm) started from 24 h from the beginning of experiment and continued every 24 h for 14 days (Jordan and Haferkamp, 1989). To maintain the moisture, the arranged Petri dishes in the tray were kept in bright nylon and 1 ml of water was added whenever the moisture decreased. Then, the germination speed was calculated using Maguire method (Equation 1) (Maguire, 1962).

$$Rs = \sum_{i=1}^{n} \frac{Si}{Di} \qquad \text{(Equation 1)}$$

Where

I and n= number of samples from 1 to n

R_s= germination speed (t number of germinated seeds in a day)

S_i= number of germinated seeds per counting and

D_i= number of days until nth counting.

Cardinal temperatures (minimum, optimum and maximum) were determined using linear regression models between germination speed (on the basis of number of germinated seeds in a day) and different temperatures considered as an independent variable (axis x) and germination speed was considered as a dependant variable(axis y)

(Wise and Binning, 1987). Intersection lines model (ISL) was developed using Equations 2, 3 and 4 (Phartyal *et al.*, 2003).

$f = if(T\ To, region1(T), region2(T))$ (Equation 2)

$Region1(T) = b(T - Tb)$ (Equation 3)

$Region2(T) = c(Tm - T)$ (Equation 4)

The five-parameter β model (FPB) was developed using Equations 5 and 6 (Zhou *et al.*, 2005).

$f = exp(\mu)(T - Tb)^{a}(Tm - T)\beta$ (Equation 5)

$T_O = \frac{(\alpha T_m + \beta T_b)}{(\alpha + \beta)}$ (Equation 6)

Where

α and β= regression coefficients,

f= seed germination speed,

T= temperature (°C),

T_b, T_O and T_m= basal, optimum and maximum temperatures, respectively.

Angular conversion was conducted for data related to germination percent (Ale Ebrahim *et al.*, 2009). The mean comparison of data was conducted using Duncan multi-ranges test at confidential level of 5%. To conduct the data analysis and draw the curves, SAS 9.1 and Excel software were used. Regression models were fitted using Write (Version 2.0) Slide software.

Results

The elementary results show that germination response of *Poterium sanguisorba* to time under different constant and alternative temperatures was different. Data achieved from the effects of thermal treatments on time passing to 50% germination, germination speed, germination percent, plunume and radicle lengths of *Poterium sanguisorba* seeds are shown in Table 1. The effects of different temperatures on seed germination speed were significant ($P \leq 0.05$). Nevertheless, the response of germination speed to different studied temperatures followed the same trend as that of germination percent, but the highest value of germination speed was not necessarily in conformity with that of germination percent. In other words, whereas the highest value of seed germination percent was obtained at 15°C, the highest germination speed occurred at 35°C. The longest time passing to 50% germination was obtained at 10°C and the greatest changes in time passing to 50% germination occurred at constant thermal range of 10 to 20°C; on the other hand, the shortest time passing to 50% germination was observed at 45°C with germination of 0 (Table 1). The effects of different temperatures on plunume and radicle lengths of *Poterium sanguisorba* were also significant ($P \leq 0.05$). The longest time passing to 50% germination at longest plunume and radicle lengths was observed at 10 and 15°C with 8.3 and 5.63 seed/ day, respectively. The longest plunume length was observed at 10°C (4.98 cm) and 15°C (4.47 cm), respectively and the shortest plunume length was observed at 35°C and 45°C (1.28 and 0 cm, respectively). The longest radicle length also occurred at 10°C and 15°C (15.3 and 5.05 cm, respectively) and the shortest radicle length was observed at 35°C and 45°C (0.88 and 0 cm, respectively) (Table 1).

Table 1. Means comparison of time passing to 50% germination, germination speed, germination percent, plunume and radicle lengths of *Poterium sanguisorba* seeds under different temperatures

Temperature (°C)	Germination Percentage	Time Passing to 50%Germination	Germination Speed (Number of Germinated Seeds in Day)	Plunume Length (cm)	Radicle Length (cm)
5	0.0 ^{c}	0.00 ^{f}	0.00 ^{f}	0.00 ^{d}	0.00 ^{f}
10	92 ^{ab}	8.30 ^{a}	2.78 ^{e}	4.94 ^{a}	5.12 ^{a}
15	98 ^{a}	5.63 ^{b}	4.58 ^{d}	4.47 ^{ab}	5.05 ^{a}
20	96 ^{ab}	4.05 ^{c}	5.93 ^{c}	2.67 ^{bc}	2.57 ^{abc}
30	87 ^{b}	3.10 ^{de}	7.77^{ab}	2.65 ^{bc}	4.2a ^{b}
35	91 ^{ab}	2.77 ^{e}	8.95 ^{a}	1.28^{cd}	0.88 ^{c}
45	0.0 ^{c}	0.00 ^{f}	0.00 ^{f}	0.00 ^{d}	0.00 ^{f}

*mean values with the same letters have no significant differences on the basis of Duncan multi-ranged test at 5%

The effects of different thermal treatments and cardinal temperatures on seed germination speed of *Poterium sanguisorba* were evaluated on the basis of intersecting lines, square equation and five-parameter β models (Table 2 & Fig. 1 (a, b, c)).

On the basis of intersecting lines method, cardinal temperatures of germination including minimum, optimum and maximum temperatures were determined to be 3.38, 34.5 and 45°C, respectively. These temperatures were 6.65, 26.82 and 46.78°C and 4.99, 32.97 and 45.03°C on the basis of square equation polynomial and five-parameter β models, respectively (Table 2). Estimations of cardinal temperatures on the basis of three studied models showed that the minimum, optimum and maximum temperatures were fluctuating within the thermal ranges of 3.38-6.65°C, 26.82-34.5°C and 45-46.78°C, respectively which conformed to each other on the basis of three studied models, but the conformity degree was more between five-parameter β and intersecting lines models.

Table 2. Values of cardinal temperatures relative to seed germination of *Poterium sanguisorba* on the basis of three fitted models

Temperature(°C)	Square Equation	Intersecting Lines	Five-Parameter β
Minimum	6.65	3.38	4.99
Optimum	26.82	34.5	32.97
Maximum	46.78	45.0	45.03
R^2	0.81	0.99	0.98

R^2 = Explanation coefficient ($P \leq 0.05$)

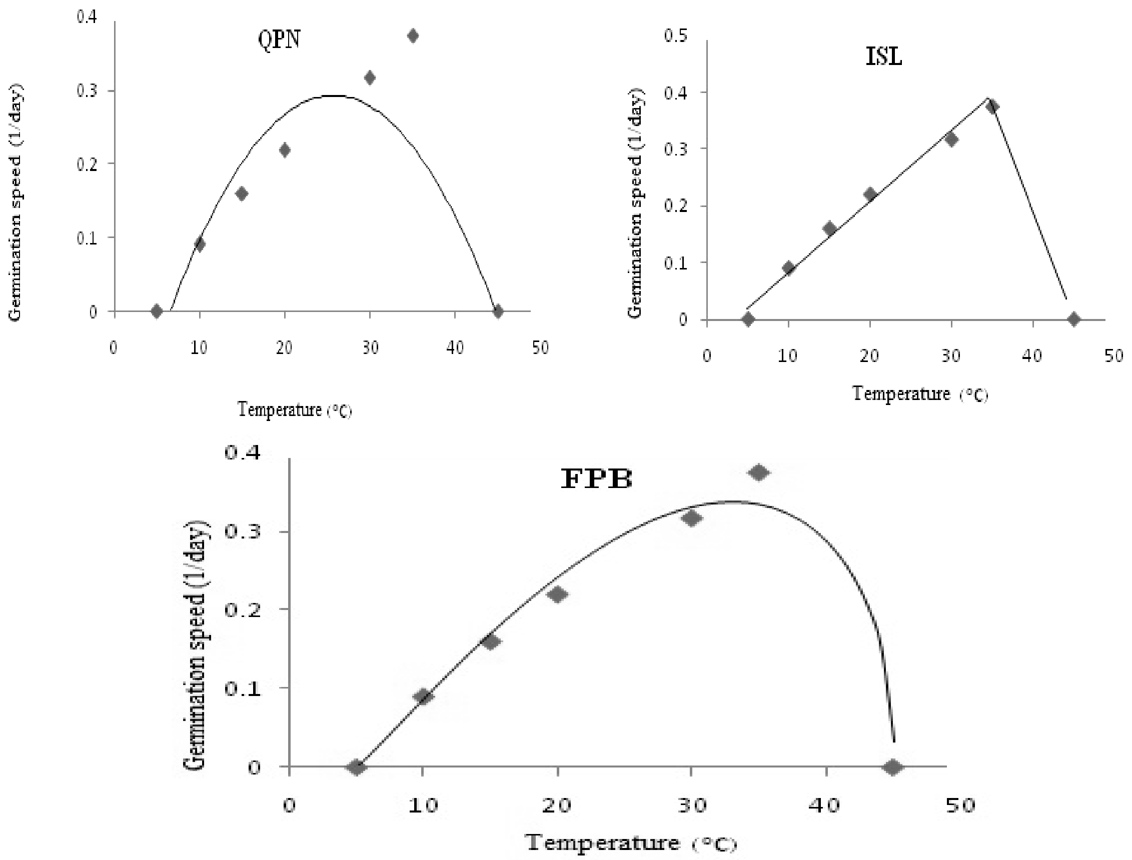

Fig. 1. Effects of different thermal treatments on seed germination speed of *Poterium sanguisorba* on the basis of intersecting lines (ISL), square equation polynomial (QPN) and five-parameter β (FPB) models

Discussion

Temperature is one of the most important factors in plant growth and development processes and plays a critical role in the regulation of plant vital processes such as seed germination (Parmoon *et al.*, 2015). The results showed that the seed germination components of *Poterium sanguisorba* are dependent on temperature. The differences between the mean values of seed germination components at different temperatures were significant ($P \leq 0.05$) with the highest seed germination percent occurring within thermal range of 10-35°C although the value was more remarkable at 15 and 20°C. Germination stopped at 5°C and 45°C. The minimum and maximum germination percent occurring at 45°C and 15°C was 0% and 98%, respectively. Studies done by Ghaderi *et al.* (2008) and Sincik *et al.* (2004) support this finding.

As there was no significant difference between thermal fluctuations and other treatments in terms of increased seed germination percent of *Poterium sanguisorba*, the plant seems to have high compatibility range and germinates easily in a wide range of environmental conditions and the germination is not sensitive to environmental conditions as suggested by Pahlavani *et al.* (2008). It can be considered as an advantage for developing the species plantation. According to the limited treatments of thermal fluctuations, it is necessary to conduct comprehensive studies on the effects of thermal fluctuations and also the effects of light and darkness on the germination behavior of plant. The results of many studies show that light can simulate the germination at low temperatures and it is believed that light compensates low temperature. Besides, it seems that seeds of *Poterium* need no alternative temperatures to germinate. But thermal alternation can cause the increase in germination. Studies also show that most weeds germinate at constant temperature and do not need alternative temperatures but their germinations increase at alternative temperatures (Leon and Knap, 2004; Zhou *et al.*, 2005). The highest germination speed was observed at 35°C and the lowest one occurred at 45°C (0%). The mean seed germination speeds within the range of constant temperatures from 5 to 45 and alternative temperatures between 15 and 30°C were 4.28 and 7.51 seed/day, respectively. Tabrizi *et al.* (2006) stated that the germination speed is a more sensitive thermal index than the germination percent that affects the plant germination. Generally, temperature affects the seed final germination percent due to the effects on dormancy, germination speed and growth rates of plunume and radicle (Bradford, 2002). Many reports are indicative of the increasing effects of temperature to a specific point on seed germination percent and speed (Bannayan *et al.*, 2006; Tabrizi *et al.*, 2006; Pourtousi *et al.*, 2008).

The germination period (from the beginning and end of germination) is an important determining factor in germination response to different treatments. The difference in time passing to 50% germination in each treatment can provide an idea for germination period. Time passing to a variety of germination percent is an index which is greatly affected by germination predominant conditions and temperature (Pourtosi *et al.*, 2008; Alvardo, 2000). The results of this study showed that the effects of different thermal treatments on seed germination of common burnet were significant with the lowest germination speed of 2.26 seeds a day at 5°C and the highest germination speeds of 8.3 and 5.63 were obtained at 10 and 15°C, respectively. Adam *et al.* (2007) declared that the seedling growth and germination response to temperature can differ interspecies and even masses in one species. For example, germination speeds of different seed masses of canola were different at 10°C but the same at 22 and 25°C (Nykiforuk and Johnson-Flanagan, 1994). It is expected that high temperatures lead to seed decline besides the decreased germination speed (Hardegree, 2006). Some studies indicate that at least within a proper thermal range, germination speed increases linearly with the increase in temperature, but it shows a great decrease in higher temperatures (Mwale, 1994). Varied germination responses to different temperatures are due to the fact that the enzyme activity and thereby enzyme activity efficiency increase with the increase in temperature followed by the improvement of germination speed. On the other hand, very low and high temperatures also lead to the inactivation of some enzymes and the decreases in the speeds of such reactions. Although the optimum and maximum germination temperatures depend on plant genetics and climatic conditions, the optimum and maximum germination temperatures for most plants have been reported between 15-30 and 30-40°C, respectively (Copland and MC Donald, 1995).

Results illustrated that the relationship between temperature and germination speed in *Poterium* can be shown by three studied models which are used to determine the cardinal temperatures of plant germination. The minimum, optimum and maximum temperatures were obtained within the thermal ranges of 3.38-6.65°C, 26.82-34.5°C and 45-46.78°C, respectively. Jami and Kafi (2007) in quantifying the relationship between temperature and germination speed using β function found that the minimum, optimum and maximum temperatures for wheat germination are 0, 30 and 42°C, respectively. Seefeldt *et al.* (2002) also showed that the basal temperatures of 6 spring wheat varieties varied between 1.2 to 1.6°C. They also showed that the germination and elongation speeds of seedlings increased linearly at 5-25°C.

Conclusion

The results of the present study confirmed that in the absence of other limiting factors such as light and water, the germination of *Poterium sanguisorba* seeds was highly influenced by temperature. As it is considered, five-parameter β (FPB) and intersecting lines (ISL) are the best fitted models in this regard. The intersecting lines provided the best fitness. So, the stated models sharply define the cardinal temperatures of *Poterium sanguisorba*.

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بررسی خصوصیات جوانه زنی بذور *Poterium sanguisorba* تحت تأثیر انواع تیمارهای دمایی به منظور احداث چراگاه

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چکیده. گیاه توت روباه (***Poterium sanguisorba***) گیاهی چندساله از تیره گل سرخ (***Rosaceae***) است، که برای احداث چراگاه‌ها استفاده می‌شود. گیاه مذکور به شرایط یخ‌زدگی، سرما و خشکی مقاومت دارد. این مطالعه به منظور بررسی خصوصیات جوانه‌زنی بذور این گیاه تحت تأثیر تیمارهای مختلف درجه حرارت در قالب طرح کاملاً تصادفی با چهار تکرار، در سال ۱۳۹۲ انجام شد. تیمارها شامل اثر دماهای ثابت ۵، ۱۰، ۱۵، ۲۰، ۳۰، ۳۵ و ۴۵ درجه سانتی‌گراد بر جوانه زنی بذر بودند که در هر تکرار از ۲۵ عدد بذر استفاده شد. نتایج نشان داد که اثر تیمارهای مختلف درجه حرارت روی خصوصیات جوانه زنی گیاه توت روباه معنی‌دار است، به طوری که کمترین سرعت جوانه‌زنی در دماهای ۵ و ۱۰ درجه سانتی‌گراد به ترتیب با ۰ و ۲/۷۸ بذر در روز و بیشترین سرعت جوانه‌زنی در دماهای ۳۵ و ۳۰ درجه سانتیگراد با ۸/۹۵ و ۷/۷۷ بذر در روز بدست آمد. بیشترین طول ساقه‌چه در دماهای ثابت ۱۰ درجه سانتیگراد (با ۴/۹۴ سانتیمتر) و ۱۵ درجه سانتیگراد (با ۴/۴۷ سانتیمتر) و کمترین طول ساقه‌چه در دماهای ثابت ۳۵ و ۴۵ درجه سانتیگراد (به ترتیب با ۱/۲۸ و ۰ سانتیمتر) مشاهده شد. بیشترین طول ریشه‌چه نیز در دماهای ثابت ۱۰ درجه سانتیگراد و ۱۵ درجه سانتیگراد (به ترتیب با ۵/۱۳ و ۵/۰۵ سانتیمتر) و کمترین طول ریشه‌چه در دماهای ثابت ۳۵ و ۴۵ درجه سانتیگراد (به ترتیب با ۰/۸۸ و ۰ سانتیمتر) مشاهده شد. با توجه به مدل‌های رگرسیون برازش داده شده بین سرعت جوانه زنی و درجه حرارت، مقادیر درجه حرارت‌های حداقل، مطلوب و حداکثر به ترتیب در دامنه ۶/۶۵- ۳/۳۸، ۳۴/۵- ۲۶/۸۲ و ۶۴/۷۸- ۴۵ درجه سانتی گراد بدست آمد. با توجه به جوانه زنی این بذر در دماهای حداکثر و حداقل می‌توان از این بذر جهت احداث چراگاه در مناطق خشک و نیمه خشک ایران استفاده کرد.

کلمات کلیدی: دما، ***Poterium sanguisorba***، جوانه‌زنی، احداث چراگاه