

GERMINATION OF THREE LEGUMES: *ONOBRYCHIS SUBNITENSE* BORNM., *ONOBRYCHIS SCROBICULATA* BOISS., AND *VICIA VARIABILIS* GROSSH. AT DIFFERENT TEMPERATURES

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Abstract. Cardinal temperatures and thermal time are valuable information for scheduling year-round production of seed-propagated species. In this study, thermal germination behavior of three species of the family Fabaceae, *Onobrychis subnitens* Bornm., *Onobrychis scrobiculata* Boiss. and *Vicia variabilis* Grossh., from Kurdistan Province, Iran were quantified. Seeds of these species were germinated at temperatures of 5–35°C. Three nonlinear regression models (segmented, beta, and dent-like) were used to estimate cardinal temperatures. After examining *O.subnitens*, findings showed that the segmented model is the best model for predicting cardinal temperatures. The base, optimum, and maximum temperatures were 1.23, 17.22 and 41.10°C, respectively. The thermal time required for 50% germination was 21.29 degree-days. For *O.scrobiculata*, segmented and dent-like models were equal. The base, optimum and maximum temperatures of seed germination were 5.5, 18.8–19.76 and 38.14°C, respectively. The thermal time required for 50% germination was 25.87 degree-days. For *V.variabilis*, there was no difference between the segmented and dent-like models and each one could be chosen. Accordingly, the base, optimum, and maximum temperatures of seed germination were -1.2, 20 and 35.13°C, respectively. The thermal time required for 50% germination was 60.9 degree-days. Based on the germination percentage, germination rate and thermal time requirement, *O.subnitens* is preferred for use in the arid and semi-arid rangeland improvement project.

Keywords: endemic species, Fabaceae, forage production, modeling, rangeland, seed dormancy

Introduction

Legumes are desirable as human and animal food, and add nitrogen to the soil if effectively nodulated. The high protein and fat content and the low content of cellulose in legumes improve the fodder value and play an important role in soil environments by increasing the nutritive worth of pastures (Abou-El-Enain, 2002). *Onobrychis* Miller (Sainfoin) which has more than 130 annual and perennial species is found in the Mediterranean region to central Asia. Most species are restricted to northwest Asia, in particular Iran and Anatolia (Yildiz et al., 1999). The productive perennial *O.subnitens* Bornm has a wide range of habitat types, is endemic to Iran (Jalili and Jamzad, 1999), and widely grown as a pasture legume (Karamian and Ranjbar, 2008). *O.scrobiculata* Boiss is

perennial and endemic to Iran (Sanandaji and Mozaffarian, 2010) has a crucial role in soil conservation and considered low-risk in terms of endangered plants in Iran (Jalili and Jamzad, 1999). The genus *Vicia* L. (Vetch) encompasses approximately 210 species (Hanelt and Mettin, 1989) and its center of diversity is the Mediterranean region (Maxted, 1993). *Vicia variabilis* Grossh. is a perennial that produces large amounts of hay and has seeds rich in protein, fat, and nitrogenous extractive substances (Mammadova and Gurbanov, 2013). This species is used as a medicinal plant in Iran (Mosaddegh et al., 2012).

Selecting regions with potential for establishment and production requires more information on environmental constraints (Adam et al., 2007). Generally, these three species play significant roles in soil conservation, forage production and rangeland rehabilitation in arid and semi-arid areas (Karamian and Ranjbar, 2008; Mosaddegh et al., 2012). An important constraint in determining the suitability of a crop for production in a region is the range of essential temperatures for germination and seedling growth.

Germination is a complex physiological process affected by different environmental factors such as temperature, light, oxygen, and moisture which has great significance in the ultimate density of the affected crop (Kharkwal et al., 2002). Temperature is a vital regulator of seed germination (Mayer and Marbach, 1981). Three cardinal temperatures (base, optimum, and maximum) indicate the range of temperature over which seeds of a particular species germinate (Bewley and Black, 1994). As the rate of germination increases between the base and optimum temperatures, decreases between optimum and maximum temperature ceases above the maximum and below the base temperatures and an estimation of the cardinal temperatures is necessary (Shafii and Price, 2001). Each species has cardinal temperatures for germination that shows its adaptation to environmental conditions (Alvarado and Bradford, 2002). Thermal-based germination models are widely used to predict germination rates and germination timing of plants. Germination rates usually increase linearly with temperature in the suboptimal range and then decrease linearly (Alvarado and Bradford, 2002). Garcia-Huidobro et al. (1982) developed a thermal time model to calculate the cardinal temperatures and the thermal time constant for both suboptimal and supra-optimal temperatures. Knowing the base temperature and thermal time constants for each species can help predict species distribution transferal under climate change (Midmore, 2015). Increases in mean global temperatures and extremes of temperature are predicted for the future (Kendall, 2012). It is predicted that in Iran, the mean annual temperature will rise by 1.5–4.5°C (Amiri and Eslamian, 2010). Therefore, one of the direct impacts of climate change is changes in temperature patterns. Plants are unable to move when conditions become unfavorable and so they have developed the ability to readily adapt their growth and developmental processes to changing conditions (Kendall, 2012).

Species vary in their germination and seedling growth responses to temperature (Adam et al., 2007; Biligetü et al., 2011; Windauer et al., 2012; Cardoso and Bianconi, 2013; Javanmard and Eshghizadeh, 2014).

The base temperature and thermal time are important, and can be used to compare germination timing between different species (Zhang et al., 2015). The thermal time model for 10 grass (Wang et al., 2011) and 12 Asteraceae (Liu et al., 2011) species at suboptimal temperatures have been investigated. Hu et al. (2012) compared methods of predicting thermal parameters in four *Vicia* species. Furthermore, although legume species play a key role in rangeland ecosystems, few studies (Hu et al., 2012; Hu et al., 2015) have evaluated thermal and hydrothermal models of legumes.

Although there is a rich body of data describing the cardinal temperatures for many agronomic crops and plant species, information on the thermal requirements of many plants is lacking. For most species, germination modeling is an effective approach in determining cardinal temperatures (Parmoona et al., 2015). Knowledge of seed germination responses to environmental factors is necessary for understanding and predicting the ecological adaptation of the species, and for formulating effective strategies for restoration (Fenner and Thompson, 2005).

This study investigates seed germination responses of *O.subnitens*, *O.scrobiculata*, and *V.variabilis* at different temperatures to establish the base, optimum, and maximum temperatures and to determine thermal time requirements which have not been previously studied. This information will be valuable for scheduling year-round production of these seed-propagated species and for providing insights into germination responses in a plant's natural habitat (Cave, 2011).

Materials and methods

Study area and seed collection

Mature fruits of *O.subnitens* and *O.scrobiculata* were collected from the wild population located between Zakha Olya and Zakha Sofla villages of Divandarre County, Kurdistan Province, Iran at the time of seed dispersal in 2014. Seeds were stored in glass bottles under laboratory conditions (RH 20–45%; 16–22°C) until used in this experiment.

Seeds of *V.variabilis* were collected from the Saral region of Divandarre County. Seeds were collected as soon as they were ripe and when they had started to disperse naturally in 2014. Seeds were stored dry under laboratory conditions (RH 20–45%; 16–22°C) in a glass bottle until used in this experiment.

The climatic and geographic characteristics of the seed collection area (Saral and Zakha) located in Kurdistan Province, Iran (*Figure 1*) are shown in *Table 1*.

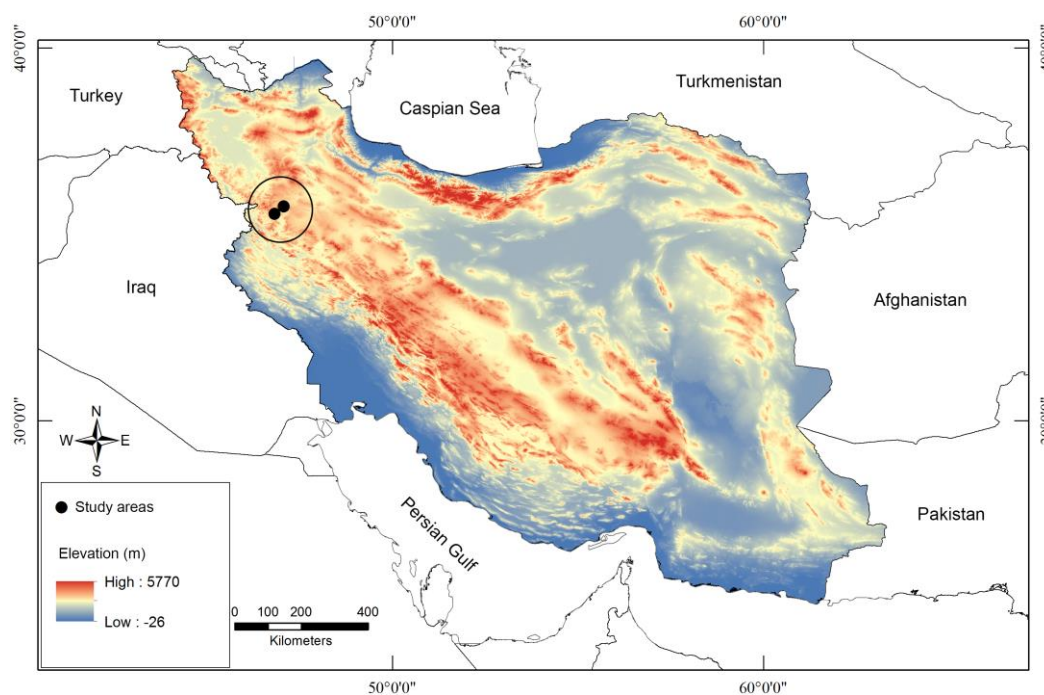


Figure 1. Map of the study area

Table 1. Environmental and Geographic characteristics of study area

Location	Annual average Temperature (°C)	Rainfall Annual (mm)	Altitude (m)	Latitude (N)	Longitude (E)
Saral	12.27	473	2170	35°30'	46°49'
Zakha	10.91	348	1786	35°45'	47°04'

Seed viability, thousand seed weight (TSW) and hard seed (physical dormancy) were determined before experiments commenced. Seed viability and TSW were determined according to the International Seed Testing Rules (ISTA, 2014).

Percentages of hard seed were determined by incubating seeds at 20°C and seeds that did not become imbibed within 14 day were considered as hard seed. Since most seeds of the three species had physical dormancy, they were mechanically scarified with different methods. No information existed on the scarification of this species and so various methods were tried (pereichiling, using hot water, removing hard shell, using concentrated sulphuric acid (98%) solution for 3 minutes, 5 minutes, 10 minutes, 15 minutes, and 30 minutes). Finally, methods with the highest germination percentage was chosen: dipping *V.variabilis* in a concentrated sulphuric acid (98%) solution for 5 minutes and washing with distilled water, removing the hard shell of *O.subnitens* fruit and then dipping in concentrated sulphuric acid (98%) solution for 3 minutes and washing with distilled water, and removing the hard shell of *O.scrobiculata* fruit.

Germination tests

After scarification, the seeds were disinfected with sodium hypochlorite for one minute followed by washing with distilled water. Four replicates of the 50 seeds were germinated in 12 cm sterilized plastic Petri dishes on top of one layer of filter paper and then moistened by adding 5 ml of distilled water. The Petri dishes were enclosed and sealed in polyethylene bags to prevent dehydration. Seeds were incubated in darkness at constant temperatures from 5–35°C (at intervals of 5°C) (Lonati et al., 2009; McCartan et al., 2015; Hu, 2015; Zhang et al., 2015). Seeds with a radicle longer than 2 mm were considered germinated. Germination counts were made daily for 15 days until the cumulative germination became stable over three consecutive days and this lasted 15 days.

Statistical analysis

The rate of germination is a more sensitive index than the germination percentage in response to temperature and moisture potential (Steinmaus et al., 2000; Khalili et al., 2013). Therefore, under normal conditions, the use of the germination rate index for different plant species to fit regression models show better results in determining cardinal temperatures (Sadrabadi Haghighi and Sazevari, 2011). Hence, the germination rate was investigated to determine cardinal temperatures. For modeling purposes, germination percentage (GP) and germination rate (R50) were calculated by using the Germin program (Soltani and Maddah-Yazdi, 2010). Germin program calculates the percentage of germination using Eq.1 (Belcher and Miller, 1974):

$$Gp = \sum (ni * 100 / N) \quad (\text{Eq.1})$$

where Gp is percentage of germination, ni is number of germinated seed/day and N is number of days that seeds were monitored.

Rate of germination was determined by Eq.2 (Hartman et al., 1990):

$$GR = \sum ni/N \quad (Eq.2)$$

where Gp is percentage of germination, ni is number of germinated seed/day and N is number of days that seeds were monitored.

For each species, a completely randomized experimental design was used with four replications. A one-way ANOVA was used to test differences in GP and R50 of 5, 10, 15, 20, 25, 30 and 35°C. Mean results were also compared by Duncan's test (performed at the 5% significance level).

There are many thermal functions to show the response of germination to temperature. Three more efficient temperature functions (beta (Eq.3), segmented (Eq.4) and dent-like (Eq.5)) were used (Soltani et al., 2006; Soltani et al., 2008).

Beta function with (β) Symbol:

$$f(T) = \left\{ \left[\left(\frac{T-T_b}{T_o-T_b} \right) \left(\frac{T_o-T}{T_c-T_o} \right) \right]^{(T_c-T_o)/(T_o-T_b)} \right\}^\alpha \quad \text{if } T > T_b \text{ and } T < T_c \quad (Eq.3)$$

$$f(T) = 0 \quad \text{if } T \leq T_b \text{ or } T \geq T_c$$

Segmented function:

$$f(T) = (T - T_b) / (T_o - T_b) \quad \text{if } T_b < T \leq T_o \quad (Eq.4)$$

$$f(T) = (T_c - T) / (T_c - T_o) \quad \text{if } T_o < T < T_c$$

$$f(T) = 0 \quad \text{if } T \leq T_b \text{ or } T \geq T_c$$

Dent-like:

$$f(T) = (T - T_b) / (T_{o1} - T_b) \quad \text{if } T_b < T < T_{o1} \quad (Eq.5)$$

$$f(T) = (T_c - T) / (T_c - T_{o2}) \quad \text{if } T_{o2} < T < T_c$$

$$f(T) = 1 \quad \text{if } T_{o1} < T < T_{o2}$$

$$f(T) = 0 \quad \text{if } T \leq T_b \text{ or } T \geq T_c$$

where T, T_b, T_o, and T_c are mean, base, optimum, and maximum temperature, respectively, T_{o1} is the lower optimum temperature (for dent-like function), T_{o2} is the upper optimum temperature (for dent-like function) based on degree centigrade. α is the shape parameter of the beta function. To determine these parameters, PROC NLIN in SAS was used. The coefficient of determination (R²), the coefficient of variation (CV), and lower deviations of the intercept from 0 and of the slope from 1 corresponding to the increased reliability (RMSE) were used to choose the best model.

To compare the model for estimating cardinal temperature, the Akaike Information Criterion (AIC) was used (Eq. 6) (Burnham and Anderson, 2002).

$$AIC = n * \ln(ss \text{ model}/n) + 2*k \quad (Eq.6)$$

where n is the number of data points (observations), Ln is the natural logarithm, RSS is the residual sums of squares and K is the number of parameters in the model.

Thermal time (θ , °C day) estimates for each species was calculated by using Eq.7 for suboptimal and Eq. 8 for supra-optimal temperatures (Bradford, 2002):

$$TT_{sub} = (T - T_b) tg \quad (Eq.7)$$

$$TT_{supra} = (T_c - T) tg \quad (Eq.8)$$

where T is the germination temperature, T_b and T_c are base and maximum temperature, and tg is the needed time to reach 50% of the germination.

Results

Effect of temperature on germination percentage

Temperature had a significant effect on both the percentage and rate of germination for all species ($p=0$). Highest germination of *O.scrobiculata* occurred between 15–20°C (more than 93%); the lowest germination percentage was at 5°C (no seed germinated). The highest germination for *O.subnitense* occurred at the temperature range of 10–20°C (more than 90%) and the lowest germination percentages at 5°C and 35°C. For *Vicia variabilis*, the optimum germination temperature was between 10°C and 20°C (more than 88%); but no seed germinated at 35°C (Figure 2).

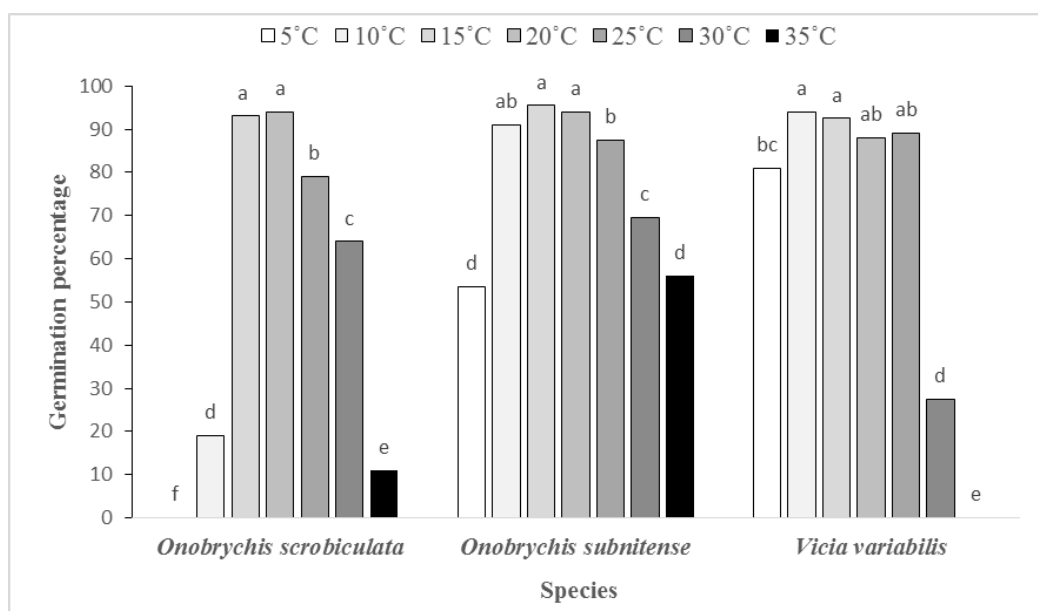


Figure 2. Germination percentage for the three species seeds affected by different temperature (values followed by different letters are significantly different ($P < 0.05$))

Effect of temperature on germination rate

The fastest germination rate for *O.scrobiculata* was observed at 20°C (10 seed/day), and the slowest rates at 5°C, 35°C, and 10°C. The maximum germination rate of *O.subnitense* was at 15°C and 20°C, and the minimum at both 5°C and 35°C. *Vicia variabilis* germinated fastest at 20°C, but at 35°C was 0 (Figure 3).

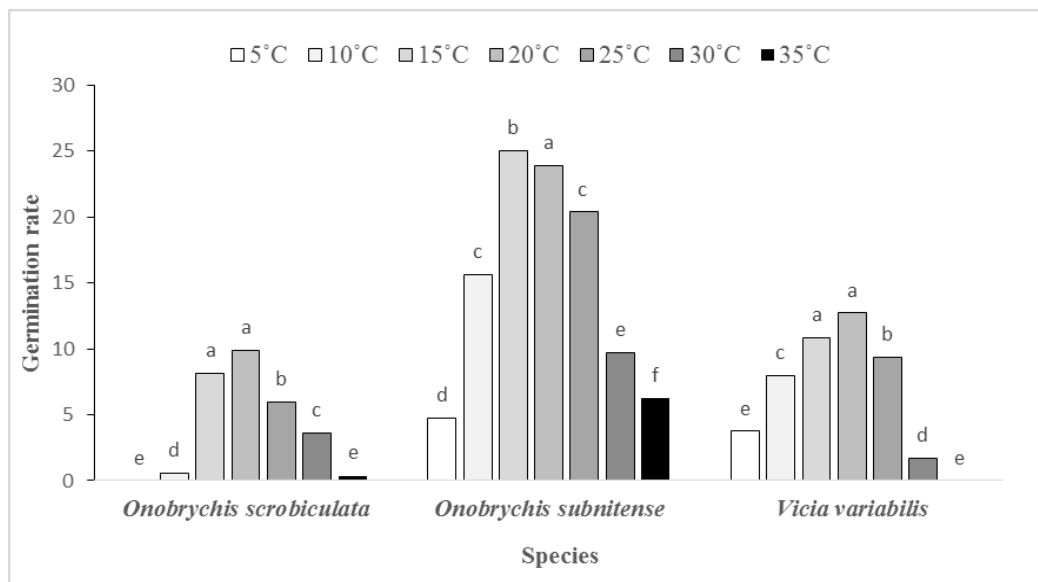


Figure 3. Germination rate for three species seed affected by different temperature (values followed by different letters are significantly different ($P < 0.05$))

Use of models to explain germination

Cardinal temperatures and maximum germination rate of the studied species based on beta, segmented and dent-like models are shown in *Table 2*. The beta and dent-like models were not useful for calculating the cardinal temperature for *O.subnitens* (P -values > 0.05 in *Table 2*), whereas the segmented model was useful. Based on the segmented model, the cardinal temperatures for the base, optimum and maximum were 1.33°C, 17.15°C and 41.83°C, respectively.

The beta model could not be used to determine the cardinal temperatures for *O.scrobiculata* (P -values > 0.05). The other indices (R^2 , RMSE, CV and AIC) for the other models (segmented and dent-like) were similar, and both models can be used to calculate cardinal temperatures. The minimum germination temperature was 5.5°C, the upper limit of the optimum temperature was 18.87°C, the lower limit of the optimum temperature was 19.69°C (optimum temperature with the segmented model was 19.22°C) and the maximum germination temperature was 38.06°C.

All three models for *V.variabilis* were acceptable (P -values < 0.05). However, the beta model is not a good model (R^2 beta model $< R^2$ dent-like and R^2 segmented model). In the dent-like and segmented models, R^2 , RMSE, and CV were similar, but the AIC index of the dent-like model was less than the segmented model. Hence, the dent-like model appears to be the most suitable model. In this model, the base temperature was -1.35°C, the lower limit of the optimum temperature was 19.44°C, the upper limit of the optimum temperature was 20.9°C, and maximum temperature was 38.06°C.

The thermal time model is described well and the relationship between germination rate and temperature ($R^2 > 0.9$) is illustrated in *Tables 3 and 4*.

V.variabilis has the highest and *O.subnitens* has lowest thermal time constant coefficient in sub-optimal temperatures.

In supra sub-optimal temperatures *V.variabilis* has the highest and *O.subnitens* has the lowest thermal time constant.

Table 2. Cardinal temperatures, maximum germination rate, significant level, R^2 , RMSE, CV and AIC of segmented, Beta and Dent like model in three species

species	model	cardinal temperatures			r max	P-value	R^2	RMSE	CV	AIC
		base	optimum	maximum						
O. subnitense	Beta	$1 \cdot 10^{-8} \pm 1$	20 ± 8.6	35 ± 7.5	0.022	0.54	0.95	0.0019	18.2	-254.51
	Segmented	1.2 ± 1.6	17.2 ± 1.1	41.1 ± 2.3	0.031	0.01	0.96	0.0018	10.2	-303.88
	Dent like	1.3 ± 1.6	15.0 ± 1.6 21.2 ± 2.8	41.05 ± 2.8	0.027	0.052	0.97	0.0014	8.23	-302.72
O. scrobiculata	Beta	$1 \cdot 10^{-8} \pm 2$	20 ± 2.2	35 ± 19.8	0.022	0.2	0.9	0.0029	28	-272.8
	Segmented	5.5 ± 0.9	19.2 ± 1.0	38.1 ± 1.5	0.023	0.006	0.97	0.0013	12	-305.59
	Dent like	5.5 ± 0.9	18.8 ± 1.46 19.7 ± 1.2	38.1 ± 1.5	0.022	0.006	0.97	0.0013	12	-303.59
V. variabilis	Beta	$-2 \pm .01$	18.9 ± 1.3	35.4 ± 4.7	0.012	0.0047	0.94	0.0012	16	-296.8
	Segmented	-1.2 ± 1.6	20 ± 0.6	35.1 ± 0.5	0.13	0.0014	0.99	0.0004	6.5	-335.63
	Dent like	-1.4 ± 1.6	21.0 ± 1.5 19.6 ± 0.5	35.1 ± 0.5	0.14	0.0013	0.99	0.0004	6.45	-333.63

Table 3. The thermal time constant coefficient (θT) for temperatures lower than the optimal temperature

species	$\theta_T \pm SE$ (° C/ day)	p	R^2	RMSE
<i>O. subnitense</i>	21.3 ± 1.2	0.3	0.99	0.0008
<i>O. scrobiculata</i>	25.8 ± 2.4	0.008	0.98	0.0016
<i>V. variabilis</i>	59.4 ± 3.2	0.002	0.99	0.0004

Table 4. The thermal time constant coefficient (θT) for temperatures greater than the optimal

species	$\theta_T \pm SE$ (° C/ day)	p	R^2	RMSE
<i>O. subnitense</i>	31.7 ± 6.1	0.03	0.92	0.0028
<i>O. scrobiculata</i>	33.9 ± 3.99	0.01	0.97	0.0015
<i>V. variabilis</i>	44.4 ± 2.9	0.004	0.99	0.0007

Discussion

Seed germination performances studied under laboratory conditions are critical to understand species distribution (Kissmann and Habermann, 2013). *O.subnitense* had the highest germination percentage and germination rate (6.4 seeds/hour) in this study. The base temperatures for *V.variabilis* was below zero. Base temperatures can be below 0°C in rangeland species (Hardegree and Van Vactor, 1999; Trudgill et al., 2000). Although *V. variabilis* has tolerance to negative temperatures, *O.subnitense* has a higher germination rate and germination percentage which means it can resist cold weather better. By increasing temperatures up to an optimum level, the rate of germination was increased and declined thereafter (Alvarado and Bradford, 2002). Hu (2015) has confirmed this result for approximately eight different plants of the Fabaceae family. The germination rate has a positive linear relationship between sub-optimal temperatures, and a negative linear relationship between supra-optimal temperatures. Previous studies have presented similar results (Garcia-Huidobro et al., 1982; Orru et al., 2012; Ordoñez-Salanueva, 2015).

A fast germination can increase the probability of timely exit radicle from seed and use of soil moisture as well as better seedling placement. As the temperature rises above the optimum temperature, the germination rate of the seeds decreases and at maximum temperature, the rate and germination percentage become zero (Evers, 1991). The germination process involves a series of biochemical interactions that are more dependent on temperature and water. Temperature affects active water absorption by seeds in a moist environment. Reduced enzyme activity at low temperatures and disrupted enzyme activity at high temperatures (desaturated three-dimensional building enzymes) are the main causes of reduced germination percentage at high and low temperatures (Maiti and Wesche-Ebeling, 2001). Copland and McDonald (1995) considered the change in essential protein germination the reason for stopping germination at maximum temperatures. Reducing the rate of germination by decreasing temperature is also partly related to the slow reduction of seed imbibition rates (Bewley and Black, 1994). Hardegree (2006) indicated that high temperatures can reduce germination rate and cause seed destruction. Species that have a higher germination rate and a higher germination percentage can resist cold weather better (Pessarakli, 1999).

A recent literature review showed that thermal time was negatively correlated with base temperature; species with low base temperatures required more thermal time to reach 50% germination and vice versa (Durr et al., 2015; McCartan et al., 2015; Midmore, 2015). The results of this study indicate that amongst the three species studied, *Vicia variabilis* with a lower base temperature had a higher amount of thermal time which is in line with the findings of Durra et al. (2015) and McCartan et al. (2015). However, *Onobrychis scrobiculata* with the highest base temperature did not have the lowest thermal time. Cave (2011) showed *Brunonia australis* has a lower base temperature and thermal time requirement, whereas *Calandrinia* sp. has a higher base temperature and greater thermal time requirement.

Conclusion

According to the results of the present study, it can be concluded that with cardinal temperature, *O.subnitense*, *O.scrobiculata*, and *V.variabilis* have adapted to their local climatic conditions (*Table 1* and *Table 2*). As a result, the best species for use in

rangeland rehabilitation projects is one which can tolerate higher temperatures. *O.subnitense* has the highest Tc (Table 2) and it can resist hot weather better. In general, species that have higher germination rates and higher germination percentages can resist cold weather better. It is recommended to apply *O.subnitense* in rangeland rehabilitation projects because of the high germination percentages and high germination rates in a vast range of temperatures.

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Conflict of interests. The authors declare no conflict of interests.

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