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Influence of Temperature and Water Availability on Seed Germination of Cicer Milkvetch (Astragulus Cicer L.) and Purple Prairie Clover (Dalea Purpurea Vent. Var. Pupurea)

Fuguang Gao[1], Lizhi Zhang[2*], Michael P. Schellenberg[3]

[1] Baotou Animal husbandry and Aquatic products promotion Service Center. 506 Gangtieda Road, BaoTou, 014010, Inner Mongolia, P. R. China

[2] Baotou City Garden and Scientific Research Institute. Qingnian Road, BaoTou, 014010, Inner Mongolia, P. R. China

[3] Semiarid Agricultural Research Centre, Agriculture and Agri-Food Canada, Swift Current, Saskatchewan S9H 3X2, Canada

ABSTRACT

Global warming may leads a decrease in plant diversity and increased risk for some plant extinction does exist. The effect of temperature and water availability on seed germination were investigated in many plants,and are two of the most important factors on seed germination,for the plant survival that can result in a loss or increase under global climate change scenarios, by affecting a plant's recruitment success. Therefore, research on how climate change affects seed germination is essential for our research and ability to predict the risk for plants. To examine the possible effect of climate change on two commonly grown legumes a greenhouse experiment was run at at AAFC-SPARC. One was an introduced legume, cicer milkvetch, and the other a native legume, purple prairie clover. Our findings were: the experiment with warmer temperature and decreased soil moisture to research seed germination ,the result is that reduction in the total both seed germination rate occurs, and showed the seed germination of purple prairie clover is better for the more stressful temperatures and water potentials examined in this experiment. For both legumes were examined on the control water potential the best temperature range is from 20°C to 30°C .

Keywords: Global warming; Legumes; Abiotic stressors; Recruitment

*CORRESPONDING AUTHOR:
Lizhi Zhang, Baotou City Garden and Scientific Research Institute. Qingnian Road, BaoTou, 014010, Inner Mongolia, P. R. China; Email: gaofuguang1314@163.com

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1. Introduction

The scientific evidence is clear : the earth's surface temperatures are warming at a pace,and it is a signals for the global climate change [1]. Global warming has raised the temperature 0.76℃ on the earth's surface,and the temperature is going on warm is predicted to increase it further by 1.8–4.0℃ [2]. Over the past year, the cycles of cooling and warming unfolded slowly, but in recent years global warming cycle is different before [3]. Global warming may be changed the total precipitation resulting in the more frequent occurrence of drought [4]. Drought is evident clearly in rain forests of Africa and South America in the past 30 years and in already dry regions such as southern Europe and western North America [5]. This will result in a serious impact on the plant distribution, and may enhance the risk of extinction of some species.

The temperature is warming of the earth's surface is altering these environmental cues and changing in temperature and precipitation [6], has a large influence on plant's distribution [7] and is critical to potential seed germination. With delayed watering, seedling emergence decreased in a temperate-woodland tree from southwestern United States [8]. In semiarid regions of the temperate zone,there precipitation has a large influence on the distribution limits of species. In a recent study of the semiarid prairie of Canadian, it have become warmer and drier over some decades [9]. Cutforth [10] in 2000 found that a further decrease of precipitation could be predicted.

The temperature, water, oxygen, light and chemical environmental factors can regulated germination [11]. The global warming has an effect on temperature and water availability which are necessary for the seed germination [12]. Seed germination level consequences is highly dependent on available soil moisture and require temperature [13]. The consequences of climate change on seed germination are complex and far-reaching [14], Germination was decreased with warmer [15]. Seed dormancy is known as a seed characteristic which defines what conditions should be met to make the seed germinate [16]. Seed dormancy and germination conditions depend on a balance between temperature and water levels [17,18]. Temperature and water stress imposed on parents influence the phenotypic expression of seed germination percentages and rates, and early growth [19,20]. In a drought, low temperatures can cause seed sterility [21] since low temperatures limit pollen-tube growth, warmer temperature may help some species which require higher temperature for the pollen-tube growth. Low temperature with high precipitation can delay or limit seed germination, and plant early seedling establishment [19,22]. Warmer temperature and drought tend to reduce seed dormancy, temperature affects cellular metabolic and growth rate, there have been many instances using high temperature treatment to terminated forest tree seeds dormancy [23].

Different species seeds or seeds from the same plant seed will germinate over a wide range of temperatures but will not germinate above or below their required temperature range. High temperatures may cause changes in the structure and dormancy of seed causing enhance seed germination [24], and lengthening the growing season and germination days [25]. Some seeds germinate may be require slightly higher or cooler than room temperature. Cicer milkvetch (*Astrugulus cicer L.*) was first introduced into the United States in 1926, and it was used in experimental plantings in the Great Plains from 1929 to 1935 [26]. Purple Prairie Clover (*Dalea purpurea*) is a long lived, a heat loving and a good all round plant, due to their deep tap roots caused it is a quite tolerant of dry conditions, so it is a carefree native perennial, and it can be mixed with other native species to create a prairie meadow [27]. And it is widely distributed throughout the south and central Prairies and Parklands with the purple species being more common.

The Canadian semiarid region has recurring drought periods [28], and the trend of decreasing precipitation [10] will increase the likelihood of this occurring. Whether it will lead to a loss or increase in seed germination are the important question for the predicted the change of decreasing precipitation, if it leads to a decrease that may increase the risk of some plant extinction, if it leads to increase that may providing a positive feed-back effect in climate change. We hypothesised that purple prairie clover would have characteristics suches higher germination temperature ability to germinate with lower water availability than cicer milkvetch, due to it mange of adaptation including more xeric environments. we tested whether temperature rising will negatively influence early and late-season legumes seed germination or will enhance germination. Cicer milkvetch and Purple Prairie Clover has desirable agronomic and feeding characteristic as forage legumes and they were spread widely in the word.

2. Materials and methods

The study was conducted at the Semiarid Agricultural Research Centre, Agriculture and Agri-Food Canada located in Swift Current, Saskatchewan (50°17'N, 117° 53'E, 825m a.s.l). The long-term annual precipitation is approximately 350 mm.

3. Experiment materials

Both legumes seeds resource were selected for germination testing: one is an early season legume (actively growing in May to June) Cicer milkvetch; another is late season legume (actively growing in July to August) Purple prairie clover. Seeds supplies indicated in Lethbridge Alberta, and harvest in same year. The pre-breeder seed was created by an equal quantity of seeds from each collection; the pre-breeder seed were stored at 5℃ in a seed storage room prior to this study.

4. Experimental test design

A randomized complete block design with two replicates was used and replicates were placed into growth chambers at three days intervals. Germination experiment considered two-factors; germination water and germination temperature. Germination water potentials controlled by osmotic potentials of 0, –0.5, –1.0 M Pa, respectively. The osmotic potentials were obtained using were made by Polyethylene glycol what was dissolved in distilled water to make solution [29], distilled water was used as control; Germination temperatures with five values ranging from 15℃ to 35℃ in 5℃ increments.

Each treatment of temperature and water potential were randomly applied to the experiment unit of 100-seed samples and were germinated at the same time in petri dishes between two layers of Whatman's NO. 2 ashless filter papers that were moistened by adding 5 ml of distilled water or Polyethylene glycol solution. The petri dishes were placed on the thermal gradient plate (TGP) at a constant temperature of each chamber with no light.

Germination counts were made daily for 21 days, seeds with a radicle greater than 3 mm were considered germinated and germinated seeds removed out of petri dishes. The experiment was repeated twice.

5. Statistical analysis

The germination index (GI) and Germination potential were calculated as follows:

$$GI = (Ti\ Ni)/S \tag{1}$$

Where Ti is the number of days after sowing, Ni is the number of seeds germination on day I, and S is the total number of seed planted [12].

$$\text{Germination potential} = Ni/N \tag{2}$$

Where Ni is the number of seeds germinated on the ith day before 7 days, and N is the total number of test seeds.

$$\text{Germination rate} = ni/N \tag{3}$$

Where ni is the number of total germinated seeds, N is the total number of test seeds.

All data analyses were conducted with SAS 9.0 statistical software (SAS 9.0, USA). A three-way analysis of variance (ANOVA) was conducted for total germination using the PROC MIXED procedure of SAS [30] with germination water, germination temperature and their interaction as fixed effects.

6. Results

6.1 Effect of varying temperature and water potential on the germination, GI and germination potential

There were statistically significant effects for water potential、temperature and their interaction with the seed germination rate, germination index (GI) and germination potential ($P < 0.05$), except germination potential for their interaction (**Table 1**). When the seed was placed in water potential at higher than 15 °C but lower than 35 °C during 7 days the seed showed rapid germination but placed in water potential –0.5 MPa and –1.0 MPa. Outside the minimum and maximum tolerated temperatures for germination are not good for seed germination. At 15 °C, Cicer milkvetch seeds germinated after two days imbibition in distilled water or PEG solution, and at 35 °C, there are just a little seed germinated in PEG solution, only reached 2% and 2.33%, respectively. Germination was significantly ($P < 0.05$) lower than in distilled water. At 20 °C and 25 °C with total, they are better for seed germination, germination of both species reaching 70%–90% (**Figure 1** and **Table 2**). The total seed germination of purple prairie clover

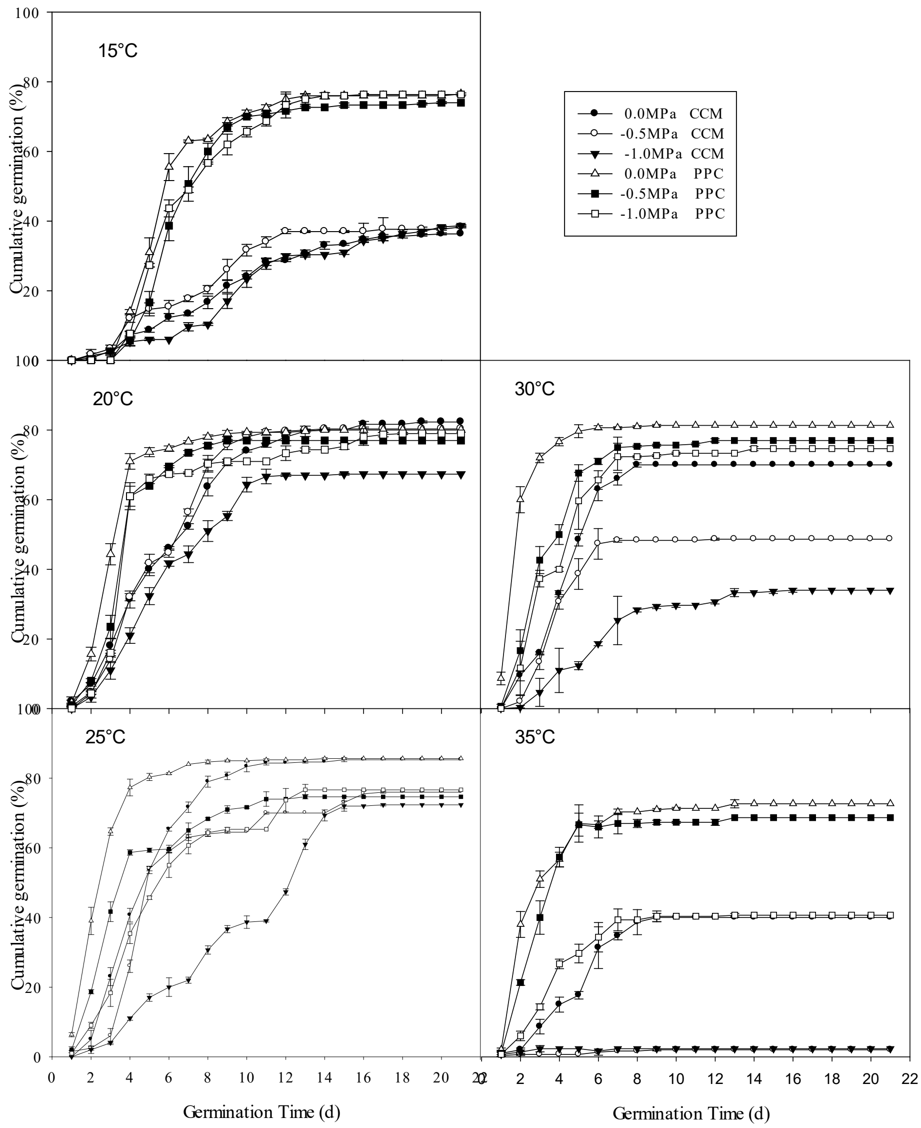

Figure 1. Germination percentage of Cicer milkvetch and Purple Prairie clover over varying of temperature and water potential.

Note: Values are mean ± SE.

was highest between 20–30 °C, lowest germination at 15 °C and 35 °C in distilled water, and at 35 °C in PEG solution, the total seed germination of cicer milkvetch showed a similar trend with the highest between 20–25 °C, and lowest at 15 and 35 °C in distilled water, at 35 °C in PEG solution (**Table 2**). Both species seeds germination rate decreased as available water decreased (**Figure 1** and **Table 2**). Cicer milkvetch seed germination rate decreased was significant except at 15 °C, while purple prairie clover seed germination rate decreased was significant at 35 °C ($p < 0.05$).

Table 1. Comparative final germination, GI and germination potential of Cicer milkvetch and Purple prairie clover under the temperature and water potential.

	Germination			GI			Germination potential		
	F value	pr > F	Sem[y]	F value	pr > F	Sem[y]	F value	pr > F	Sem[y]
Spe [z]	229.27	< 0.0001	1.05	431.69	< 0.0001	0.38	11.01	0.0010	2.70
Water	58.28	< 0.0001	1.29	102.38	< 0.0001	0.46	4.69	0.0106	3.40
Temp	126.98	< 0.0001	1.67	96.84	< 0.0001	0.67	2.95	0.0222	4.41
spe*water	4.77	< 0.0098	1.83	2.47	0.09	0.66	0.38	0.6823	4.84
spe*Temp	25.89	< 0.0001	2.36	11.58	< 0.0001	0.85	0.48	0.7504	6.24
water*Temp	6.31	< 0.0001	2.89	6.88	< 0.0001	1.04	0.31	0.9596	7.65
spe*water*Temp	4.05	< 0.0001	4.10	1.98	0.05	1.48	1.49	0.1665	10.82

Notes: [Z]Spe, species; Temp, temperature; water, water potential.

Sem [y] standard error of the means.

Table 2. Final germination rate (%) of Cicer milkvetch and Purple prairie clover under the temperature and water potential.

	CCM			PPC		
	Water potential (MPa)			Water potential (MPa)		
Temperature(°C)	0.00	-0.50	-1.00	0.00	-0.50	-1.00
15.00	36.33b ± 1.03	38.33b ± 0.30	38.67b ± 0.30	76.50ab ± 0.60	74.00a ± 0.30	76.33a ± 1.49
20.00	82.33a ± 1.49	80.33a ± 0.30	67.33a ± 0.0	80.00a ± 0.30	77.00a ± 0.00	79.00a ± 0.50
25.00	85.33a ± 0.30	76.00a ± 0.00	72.33a ± 1.49	85.67a ± 0.00	74.66a ± 0.60	76.67a ± 1.55
30.00	70.00a ± 0.00	48.67b ± 0.00	34.00b ± 1.15	81.33ab ± 0.00	77.00a ± 0.00	74.67a ± 0.00
35.00	40.00b ± 0.19	2.00c ± 0.00	2.33c ± 0.00	72.67b ± 1.19	68.67b ± 1.24	40.67b ± 0.52
P	p < 0.05	p < 0.05	p < 0.05	p < 0.05	p < 0.05	p < 0.05

Notes: CCM, Cicer milkvetch; PPC, Purple Prairie Clover.

a-c Means with the same letter within columns are significantly different at $p < 0.05$ level.

6.2 Compare seeds germination, GI and germination potential in varying temperature and water potential between Cicer milkvetch and Purple Prairie Clover

There were statistically significant effects ($P < 0.05$) from varying temperature and water potential between cicer milkvetch and purple prairie clover for total germination, germination index and germination potential, including their interaction for the final germination and GI, but between 20–25 °C, there were no significant different on the germination potential, total germination and GI (**Figure 1** and **Table 1**). And there was a clear contrast in the response to varying temperature and water potential between the species. At 15 °C, 30 °C and 35 °C, the total germination, GI and germination potential of purple prairie clover are significantly ($p < 0.05$) higher than cicer milkvetch in distilled water or PEG solution (**Figure 1** and **Table 2**). The total germination of purple prairie clover is also significantly ($p < 0.05$) higher than cicer milkvetch at 15 °C, 30 °C and 35 °C. Purple prairie clover has better seed germination than cicer milkvetch in varying temperature and water potentials. Purple prairie clover is quite tolerance of high osmotic potential in dry and high temperature. The total germination of purple prairie clover and cicer milkvetch equations are normally distributed for the varying temperature (**Table 3**), the logical equation provided a good fit to the germination data from varying temperature under different water potential, it was also showed that cicer milkvetch seed germination was inhibited, and it does not germinated in lower than –0.5 MPa and a temperature higher than 35 °C, only 2%. Purple prairie clover germination occurred when the temperature higher than 35 °C and lower than 15 °C in lower water potential. But both species legumes do not germinate well enough at temperature above 35 °C.

Table 3. Equations and coefficients of determination (R^2) for the regression of seed germination on the varying of temperature in different water potential.

Species	Water	Equations	R^2	P
Cicer milkvetch	–1.0 Mpa	y = -11.72x2 + 59.754x-7.386	$R^2 = 0.9496$	$P < 0.001$
	–0.5 Mpa	y = -14.31x2 + 75.428-19.8	$R^2 = 0.9806$	$P < 0.001$
	0 Mpa	y = -12.166x2 + 22.5-20.87	$R^2 = 0.9599$	$P < 0.001$
Purple prairie clover	–1.0 Mpa	y = -5.215x2 + 23.725x + 55.658	$R^2 = 0.9110$	$P < 0.001$
	–0.5 Mpa	y = -1.284x2 + 6.639x + 68.474	$R^2 = 0.7411$	$P < 0.001$
	0 Mpa	y = -2.452x2 + 14.08x + 63.968	$R^2 = 0.9095$	$P < 0.001$

7. Discussion

Soil temperature is usual the main factor affecting seed germination when the moisture is not limiting. Hill and Luck [31] in 1991 found that there were differences among 10 legume species legumes for seed germination time. High temperature and low water availability can inhibited seeds in a manner similar to that reported using PEG osmatic [32]. The germination under high temperature maybe reached if the water is available, it is more difficult to predict germination under low available water. Extrapolating using equations and coefficients for the regression of both legume species of seed germinated poorly when the temperature was lower than 15 °C and higher than 35 °C, water potential at or lower -0.5 bars. Warmer temperature, exceeding 25 °C, decreased germinations as did lower water potentials. Purple prairie clover had a greater number of germination than cicer milkvetch at the higher temperatures and greater water potentials suggesting better adaption to the predicted future growing environment. Stout [33] is also clear certificates from *Medicago stativa* is that a large range in dural temperature variation plus a warm temperature inhibited seed germination. Species adapted to a wide range of temperature such as legumes species should germinate successfully over a wide range of temperature and be easier to establish [34].

Temperature is thought to be an important environmental variable that influences the seeds germination [35], and water also is usually the limiting factor for the germination of seed, affecting the total germination. Germination is highly dependent upon available soil moisture [13,14], as our research results demonstrate different levels of available water significantly affected seed germination. The seed osmotic potential is always negative can inhibits seed germination because seeds take up available water is difficult from drier conditions, such as our research at 35°, –0.5MPa and –1.0MPa seeds cannot germinate, lower than –0.5 MPa water potential can cause significant decrease in the germination. Seeds can be germinated under great available soil moisture and increase the chance of greater germination and seedling emergence, such as purple prairie clover can germinate under drier condition, but cicer milkvetch is difficult to germinate in extremely limited water availability. This condition indicates that purple prairie clover can germinate under low water availability found in the Canadian prairies, and it is relatively easy to establish. The seeds that germinate under drier conditions may be adapted to the environment and have greater germination under low water potential

[36].

We can saw that seeds germinated in response to changes in temperature warming, increase or reduce available water levels, and resulting interaction. Osmotic priming has been used to promote early and synchronous germination of seed in improving legumes stand establishment through management and breeding [37]. Previous seeds germination studies found that cicer milkvetch seed germination was reduced fewer than 20 °C and higher than 35 °C. Cicer milkvetch has been subjected to recurrent selection for improving seed germination at temperatures as low as 20 °C and has significant improvement possible in the total seed germination within 7 days under great conditions [37]. In this study, purple prairie clover germinated well across a wide range of temperature and osmotic pressure. And there was no differ on total germination and germination rate from 15 °C through 30 °C under the same water limiting. It was certificate that purple prairie clover able to germinated well across a wide range of temperature under the global climate change where a wide range of temperature and soil moisture change occurs [34]. Higher temperature and lower available water inhibited germination, such as our research that the total germination of cicer milkvetch under 35 °C and –0.5 Mpa, –1.0 Mpa treatment could be applied for up to 21 days just has germinated around 2%.

8. Conclusion

As global climate changes to a warmer environment with less water available, purple prairie clover has characteristics better suited to such an environment. This is in agreement with our hypothesis. This research identifies there are species of legumes presently used that may be better adapted to future climatic conditions than others.

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