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Research Article

Water and Low Temperature Applications Affects Germination and Seedling Properties of Fenugreek

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Abstract

Fenugreek (*Trigonella foenum-graecum* L.) is an annual legume traditionally used as a spice, dye, forage, medicinal etc. It includes high crude protein and nitrogen fixation in the soil. The objective of this study was to assess the effect of excess water and low temperature applications on germination and seedling properties of fenugreek. In the experiment, shoot height and root length, shoot and root dry matter, shoot and root electrical conductivity (EC) and shoot and root pH were examined. The germination and seedling properties of fenugreek were affected by the water regimes. As the temperature increased, germination and seedling properties of fenugreek were affected positively compared to the lower temperatures. Excess water and temperature application interactions were significant for the most of investigated parameters. As a result of this study, excess water and low temperature applications were a stress source but has not detrimental effect on seedling properties of fenugreek.

Keywords: Temperature, water regimes, germination, seedling, electrolyte leakage

Düşük Sıcaklık ve Su Uygulamalarının Çemenin Çimlenmesi ile Fide Özelliklerine Etkileri

Özet

Çemen (*Trigonella foenum-graecum* L.) ilaç sanayi, baharat, boya sanayi, kaba yem vb. gibi amaçlar için kullanılan tek yıllık bir baklagil türüdür. Yüksek protein içeriğine sahip ve havanın serbest azotunu fikse etmektedir. Bu çalışmanın amacı düşük sıcaklık ve su uygulamalarının çemenin çimlenmesi ve fide özellikleri üzerine etkilerini değerlendirmektir. Araştırmada gövde ve kök uzunluğu, gövde ve kök kuru madde üretimi, gövde ve kök elektriksel iletkenlik ile gövde ve kök pH içerikleri incelenmiştir. Çimlenme ve fide özellikleri su uygulamasından etkilenmiştir. Sıcaklık uygulamasının artmasıyla çimlenme ve fide özellikleri düşük sıcaklıklarla karşılaştırıldığında pozitif olarak artmıştır. Sıcaklık ve su interaksiyonu incelenen parametrelerin büyük çoğunluğunda önemli olmuştur. Sonuç olarak, düşük sıcaklık ve su uygulaması bir stres kaynağı olup, çemen bitkisinin çimlenmesi ve fide gelişimi üzerine öldürücü bir etkiye sahip olmadığı belirlenmiştir.

Anahtar Kelimeler: Sıcaklık, su uygulaması, çimlenme, fide, elektriksel iletkenlik

Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual legume species that used different purposes such as medicine, food, coffee, insect control, perfume, fodder etc. in many countries. Fenugreek seeds are especially important for pastirma (beef bacon) industry in Turkey. It is also an important alternative crop mainly cultivated roughage and silage, and improve soil fertility as a nitrogen fixer or green manure (Mehrafarin et al., 2011). Suitable management application such as sowing time, fertilization, irrigation and etc. improve plant production. Especially, sowing time is the most important under dryland condition in continental climate. Seed is essential to life because of providing a magnificent array of food,

medicine, fiber, shelter and etc. for human being. Sowing time is the most important determining factor on yield in spring crops affected longevity of growing period. In general, as sowing time delayed duration of growing period decreases but low temperature and excess water in seed bed are the main obstacle in early sowing under continental climate areas.

Germination is a complicate physiological process under the control of environmental factors such as temperature, water and light. These factors affect germination separately or combined (Shaban, 2013). Suitable temperature and water is essential to beginning of enzyme activation and consequently rapid imbibition and germination (Baskin and Baskin, 2001). Temperature affects membrane permeability and cytosolic acid production in the seed (Biligetu et al., 2011). Germination generally increases with increasing temperature until optimum temperature degrees. The optimum temperature for germination changes between 10 and 20 °C for cool seasons species (Baskin and Baskin, 2001). Within this temperature range the germination rate is the fastest but germination can begin in the lower temperature in most cool seasons species. However, lower temperature can stimulate seed germination of same plant species under lower water stress condition (Shaban, 2013).

Water is basic requirement for germination. After imbibition enzyme activation begins thereafter the other phases of germination occurs (Fenner and Thompson, 2005). The speed of enzyme activity and germination are determined by temperature interaction that occurs frequently during germination processes (Fenner and Thompson, 2005). After water content reach to critical levels during imbibition, physiological processes begin in seeds. The time of this period changes between 0-50 hours depend on plant species, seed size and temperature (Fenner and Thompson, 2005).

Most species are generally sown at the spring in the cool areas where prevails continental climate. Fenugreek is typical spring sown plant in this condition. In early spring sowing encountered low temperature and exceed water due to snow melting and high precipitation in the end of winter. To understand the response of fenugreek seed germination to lower temperature and exceed water is important to establish good stand. For this reason, the objective of this study was to examine some germination characteristics of fenugreek seeds under different temperature and water levels.

Materials and Methods

The experiment was conducted under the growth chamber condition at the Faculty of Agriculture, Ataturk University Erzurum during 2015. Fenugreek seeds were used to examine the effects of temperature (3, 6, 9 ^{0}C) and water (1 times per week, 2 times per week, 3 times per week, 4 times per week) regimes on germination and seedling growth characteristics. The soil received water up to saturation level in wet-day treatments in order to stimulate excess water condition. Total 25 fenugreek seeds were sown in sand filled pots in the size of 20 x 20 cm on December 2015, thereafter these pots were placed in a growth chamber set as above mentioned temperature range for 30 day. The experiment was arranged as a completely randomized design with five replications. The pots were watered using distilled water during the experiment period. Fertilizer or pesticides were not applied to the pots during the experiment. The number of emerged seedlings was observed every day after sowing and seedling were considered as emerged when cotyledons were visible.

At the end of experiment, 10 seedlings of 25 were randomly chosen from each replication. Shoot height were measured cut at ground level. Seedlings root were separated from the soil by washing with distilled water and the length of the roots were measured. Thereafter, shoots and roots were oven-dried at 70 ^{o}C until reaching a constant weight and the total weight was determined and it divided by 10 to estimate per seedling values.

Leachate conductivity and pH of shoot and root were determined by using deionized water. Ten seedlings were soaked in 20 mL deionized water and kept in a dark condition incubator at 25 ^{0}C for 24 hour. Electrical conductivity (EC) and pH of solutes were determined by using an EC and pH meter. The EC expressed as mS^{-1} g^{-1} (Anand et al., 2011). All data was subjected to analysis of variance based on general linear models for factorial arrangement of treatments in a completely randomized design using the Statview statistical package (SAS Institute, 1998). Means were separated using the Tukey Multiple Range Test.

Result and Discussion

The increases in germination ratio occurred after 6 days at 9 ^{0}C while it was recorded after 12 days the other temperature applications. It clearly occurred that lower seed bed temperature causes a seriously delaying in the germination of fenugreek seeds (Figure 1a). Cumulative germination percentage increased with increasing temperature. The seed received 9 ^{0}C temperatures

and reached maximum germination at 18 days. Whereas cumulative germination percentage of seeds treated 3 or 6 ^{0}C germination media temperature reached maximum value at the end of experiment.

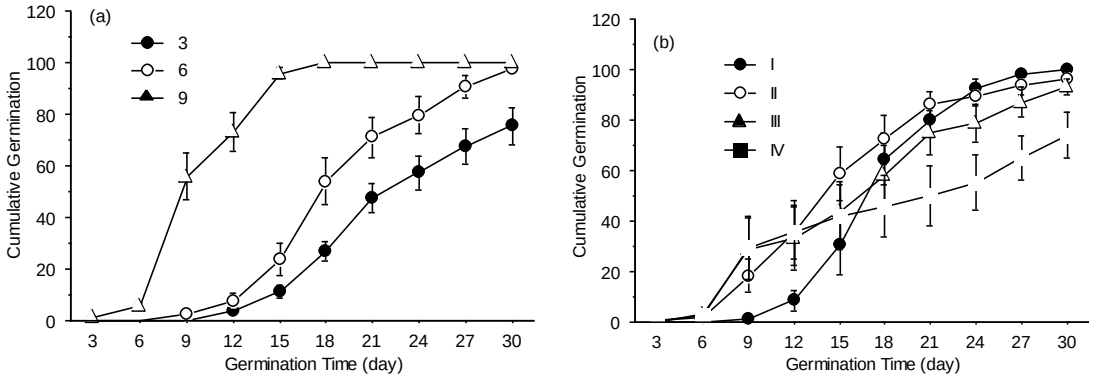

Figure 1. Cumulative germination of fenugreek a range of temperature (a) and water (b) regimes.

The seed received one day excess water per week and begin to germination after 9 days and then germination ratio increased sharply whereas the other seeds begin to germination after 6 days and was slower compared to one day excess water application (Figure 1b). The results showed that excess water cause decreases in germination rate of fenugreek seed and it was pronounced at 4 day excess water application per week treatment.

As the temperature increase shoot height increased significantly ($p<0.001$) but water application effects were different than temperature affects. The lowest shoot height was recorded at 1 times water application in week (Table 1). The increases after 2 times water application in week did not cause considerable increases in shoot height. Excess water application caused decreasing trend in shoot height, while shoot height increased with increasing water application at 9 ^{0}C temperature treatment. Therefore, water x temperature interaction was significant for shoot height (Figure 2).

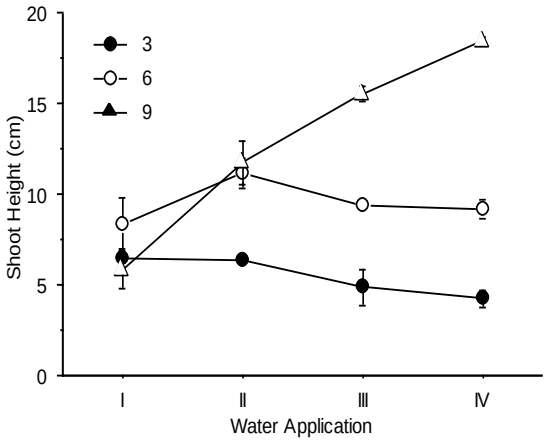

Figure 2. Seedling shoot height of fenugreek a range of water and temperature regimes.

The seedlings received 2 or more excess water per week had longer root length than seedlings received only 1 excess water treatment. 2 or more excess water applied treatments has similar root length in the experiment (Table 1). But the response of root length to excess water application changed depending on temperature regimes. At the 9 ^{0}C temperature, root length increased in line with increased excess water application whereas excess water had not a significantly positive effect, except for 2 times excess water application per week at 6 ^{0}C treatment, on root length under lower temperature condition. Hence, water x temperature interaction was significant for root length (Figure 3).

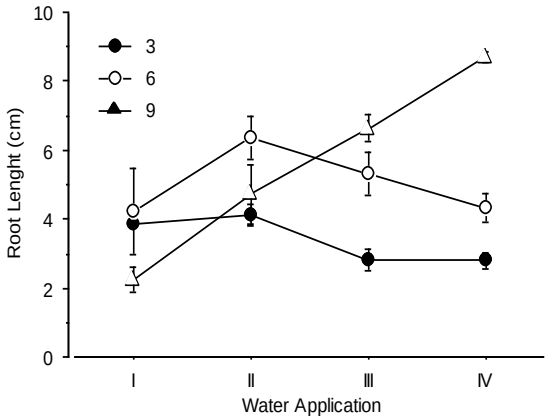

Figure 3. Seedling root length of fenugreek a range of water and temperature regimes.

An average shoot weight was 0.719 g per plant and it was changed insignificantly between 0.664 and 0.768 g per plant among water treatments and between 0.661 and 0.765 gr per plant among temperature applications (Table 1).

But water and temperature interaction was significant for shoot dry weight. The plant grown at 3 or 6 ^{0}C did not show any response to water applications while the plant grown at 9 ^{0}C affected positively 1 and 2 times excess water applications. These different responses were responsible for water x temperature interactions (Figure 4).

An average root dry matter weight was 0.130 gr per plant and the highest value was recorded at 4 times excess water applications compared to the other water applications (Table 1). Root dry weight was lower at 6 ^{0}C temperature applications than the others. There was no interactive effect of water and temperature application on root dry matter production.

Table 1. Effect of water and temperature regimes on germination and seedling characteristics after 30 days

	Shoot Height (cm)	Root Length (cm)	Shoot Dry Matter (gr/plant)	Root Dry Matter (gr/plant)	Shoot EC (mS^{-1} g^{-1})	Root EC ($mS^{-1}g^{-1}$)	Shoot pH	Root pH
Water (W)								
I	6.85 B	3.45 B	0.710	0.106 B	167.50 B	70.83 B	5.35 B	5.56 B
II	9.76 A	5.07 A	0.664	0.116 B	255.00 A	120.67 A	5.56 A	6.00 A
III	9.92 A	4.93 A	0.768	0.124 B	189.67 B	125.58 A	5.46 AB	5.98 A
IV	10.63 A	5.26 A	0.733	0.173 A	201.42 AB	110.68 AB	5.31B	5.89 A
Average	**9.29**	**4.68**	**0.719**	**0.130**	**203.40**	**106.94**	**5.42**	**5.86**
Temperature (T)								
3^{0}C	5.47 C	3.41 B	0.730	0.144 B	141.06 B	64.69 C	5.14 B	5.61 C
6^{0}C	9.51 B	5.05 A	0.661	0.091 B	397.69 A	99.31 B	5.60 A	5.76 B
9^{0}C	12.88 A	5.58 A	0.765	0.154 A	71.44 C	156.81 A	5.52 A	6.20 A
Average	**9.29**	**4.68**	**0.719**	**0.130**	**203.40**	**106.94**	**5.42**	**5.86**
W	**	**	**ns**	**	**	**	**	**
T	**	**	**ns**	**	**	**	**	**
W x T	**	**	*	**ns**	**	**ns**	**ns**	**

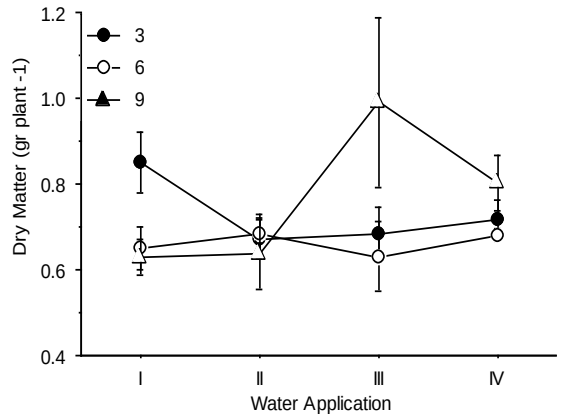

Figure 4. Seedling dry matter production of fenugreek a range of water and temperature regimes.

The water when applied excessively 2 times per week had a stronger effect on shoot leachate conductivity of fenugreek than the other water applications ($p<0.001$) (Table 1). The effect of temperature on shoot leachate conductivity was in consistent with temperature increases in germination media. The leachate conductivity increased sharply when the temperature increased from 3 to 6 ^{0}C in growth media but it decreased sharply when the temperature was increased to 9 ^{0}C. Shoot leachate conductivity of the plant grown at 6 ^{0}C was higher than the other temperature treatments at all water treatments. The leachate conductivity increased when excess water application increased from 1 to 2 and then it did not change considerably at 6 ^{0}C growth media temperature. Whereas leachate conductivity decreased after 2 times excess water application in the other temperature treatments. These different responses caused water x temperature interaction for shoot leachate conductivity (Figure 5).

The effect of water and temperature application had a significant effect on root leachate conductivity ($p<0.001$). The highest root leachate conductivity was measured at the plants received 3 times excess water per week (125.58 mS^{-1} g^{-1}) (Table 1). Root leachate conductivity increased with increased growth media temperature. While the lowest leachate conductivity was recorded at 3 ^{0}C, it was highest at 9 ^{0}C growth media temperature. Overall there was no interactive effect of water and temperature application on root leachate conductivity.

Shoot leachate pH was significantly affected by water and temperature applications ($p<0.001$) (Table 1). Average shoot leachate pH was 5.42 and

it was higher in the 2 times excess water treatments than the others. The temperature was increased from 3 to 6 ^{0}C leachate pH increased significantly but additional increases in the temperature had not significant effect. There was not interaction effect of water and temperature application on leachate pH.

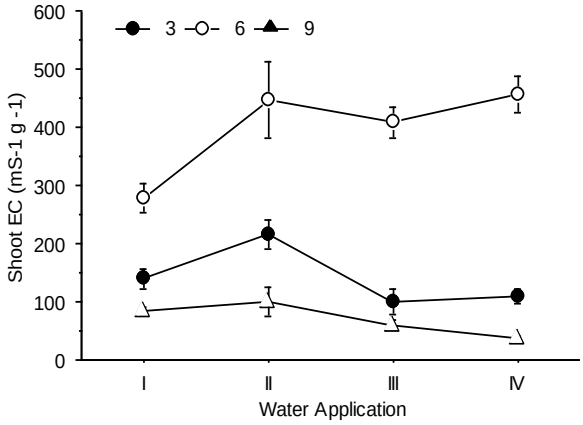

Figure 5. Seedling shoot leachate conductivity of fenugreek a range of water and temperature regimes.

All the main effects as well as the interaction were significant ($p<0.001$) for root leachate pH (Table 1). The root leachate pH was lowest at 1 times excess water applied per week than the other applications. The results showed that growth media caused that root leachate pH increases in the experiment. As the growth media temperature increase root leachate pH increase regularly in the experiment. The response of root leachate pH to excess water application at 9 ^{0}C growth media temperature was different than the other treatments. The responses of root leachate to excess water applied were not changed significantly at 3 or 6 ^{0}C growth media temperature, whereas root leachate pH was lower considerably at 1 times excess water applied plants at 9 ^{0}C root media treatments. Hence, water x temperature interaction was significant for root leachate pH (Figure 6).

The results of the experiment indicated that excess water and lower temperature application strongly affected germination and seedling characteristics of fenugreek. It is well known that water and temperature are the most important environmental factor controlling plant growth (Baskin and Baskin 2001), thus most of investigated properties were affected significantly by water and temperature applications. Seedling shoot height increased linearly in line with increased temperature. This is expected results because the temperature applied in the experiment was below the optimum temperature. Similar results were also recorded for soybean (Leopold, 1980), maize (Miedema et al., 1987), *Stipa neaei* (Bonvissuto and Busso, 2007), fenugreek (Mehrafarin et al., 2011), side-oats grama (Biligetu et al., 2011), *Elymus nutans* (Fu et al., 2017). In lower temperature, there were no significant response to excess water application with respect to shoot and root elongation in the experiment but seedling height increased in line with excess water application at 9 ^{0}C application (Figure 1 and 2). This was probably originated from increases in water use by plant and evaporative lost because water use and lost increase in line with temperature (Miedema et al., 1987; Fenner and Thompson, 2005; Bonvissuto and Busso, 2007).

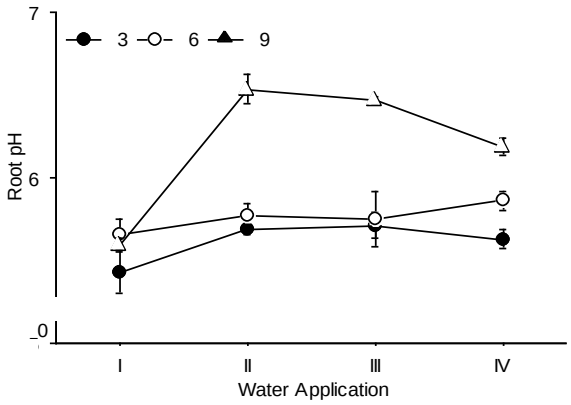

Figure 6. Seedling root leachate pH of fenugreek a range of water and temperature regimes.

Water and temperature interaction were significant for shoot dry matter production. Especially, the seedling received three times excess water per week under 9 ^{0}C temperature treatment enhanced more shoot dry matter accumulation compared to other treatments. This result showed that biomass accumulation increase in line with increased temperature under low temperature conditions and over excess water cause decrease in dry matter accumulation (Figure 3). This situation probably was originated from changing dry matter partition models in the plant. Because under excess water (4 times per week) conditions considerably increases in root dry matter production in the plants (Table 1). As is reported by Miedema et al. (1987) dry matter partition models can be changed by climatic condition especially water and temperature regimes. The decreases in root dry matter production at 6 ^{0}C temperature treatment compared to 3 ^{0}C and 9 ^{0}C was surprise and it was an unexpected result.

Conclusion

The analysis of electrical conductivity and pH of solute leakage from seedling of fenugreek have been used the barrier effectiveness of membranes or inference of seedling health. In general, increases in EC and pH are inferenced as an indicator of excess water and low temperature stress (Bramlage et al., 1978; Leopold, 1980; Leopold et al., 1981). This situation mainly stemmed from some ions exudation from tissue. In the experiment shoot and root solute leakage EC increased in line with increasing excess water application compared to 1 excess water application. This is infered as excess water cause stress in fenugreek seedling growth. As temperature increase EC and pH of solute leakage increased in the experiment. These increases were more pronounced at shoots and root solute leakage at 6 ^{0}C and 9 ^{0}C, respectively. This situation can be interfered slightly increases in excess water and temperature have side effect on seedling growth of fenugreek. Consequently, slightly increases in temperature under excess water condition can trigger stress in fenugreek but it is not detrimental for growth. Thus, it is needed to apply excess water during early growth stage of fenugreek in the spring which lower temperature prevails.

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