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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Germination and Seedling Properties of *Lotus corniculatus* L. Under Simulated Drought Stress

Simüle Edilmiş Kuraklık Stresi Altında *Lotus corniculatus* L.'nin Çimlenme ve Fide Özellikleri

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Abstract

Drought is an important abiotic stress factor that reduces agricultural production and yield in many crops, including forage crops, in agricultural areas around the world. *Lotus corniculatus* L. is the agriculturally crucial perennial legume forage crop that can tolerate moderate drought. However, studies to determine the responses of *L. corniculatus* to drought are limited. Therefore, this study was carried out to determine the seed germination and early seedling growth properties of *L. corniculatus* at different PEG_{6000} induced-drought treatments under *in vitro* conditions. In order to do this, *L. corniculatus* (cv. 'AC Langille') seeds were planted in MS (Murashige and Skoog/Gamborg) medium containing 0%, 4%, and 8% (w/v) PEG_{6000} for 14 days. In this study, germination percentage, mean germination time, germination rate index (speed of germination), shoot and root length, root to shoot length ratio, shoot and root fresh weight, shoot and root dry weight, shoot and root dry matter ratio, root shoot dry matter ratio, shoot and root water content and seedling vigor index parameters were measured. Our results showed that increasing drought levels resulted in an overall significant reduction in germination and seedling growth parameters except shortened mean germination time (especially, 4% PEG_{6000} treatment) and increased shoot and root dry matter ratio at higher (especially, 8% PEG_{6000} treatment) drought levels. When important growth parameters such as length, fresh and dry weight, dry matter ratio and water content, which show the development of root and shoot organs, are evaluated together, it has been determined that the root is negatively affected by drought stress at a higher rate. Based on these data, it can be concluded that the *L. corniculatus* will suffer a high yield loss under the drought stress at the osmotic potential (-1.03 bar) created by 8% PEG_{6000} treatment.

Keywords: *Lotus corniculatus* L., Drought stress, Germination, Seedling properties, *in vitro*

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Öz

Kuraklık, dünyadaki tarım alanlarında yem bitkileri de dahil olmak üzere birçok üründe tarımsal üretimi ve verimi azaltan önemli bir abiyotik stres faktörüdür. *Lotus corniculatus* L., orta derecede kuraklığı tolere edebilen, tarımsal açıdan çok önemli bir çok yıllık baklagil yem bitkisidir. Bununla birlikte, *L. corniculatus*'un kuraklığa verdiği tepkileri belirlemeye yönelik çalışmalar sınırlıdır. Bu nedenle bu çalışma, *L. corniculatus*'un farklı PEG_{6000} kaynaklı kuraklık uygulamalarında *in vitro* koşullarda tohum çimlenmesi ve erken fide büyüme özelliklerini belirlemek amacıyla yapılmıştır. Bunu yapmak için *L. corniculatus* (cv. 'AC Langille') tohumları, 14 gün boyunca %0, %4 ve %8 (w/v) PEG_{6000} içeren MS (Murashige ve Skoog/Gamborg) ortamına ekilmiştir. Bu çalışmada çimlenme yüzdesi, ortalama çimlenme süresi, çimlenme oranı indeksi (çimlenme hızı), sürgün ve kök uzunluğu, kök sürgün uzunluğu oranı, sürgün ve kök yaş ağırlığı, sürgün ve kök kuru ağırlığı, sürgün ve kök kuru madde oranı, kök sürgün kuru madde oranı, sürgün ve kök su içeriği ve fide canlılık indeksi parametreleri ölçülmüştür. Sonuçlar, artan kuraklık seviyelerinin, kısalan ortalama çimlenme süresi (özellikle %4 PEG_{6000} uygulamasında) ve daha yüksek sürgün ve kök kuru madde oranı (özellikle, %8 PEG_{6000} uygulamasında) dışında çimlenme ve fide büyüme parametrelerinde genel olarak önemli bir azalmaya yol açtığını göstermiştir. Kök ve sürgün organlarının gelişimini gösteren uzunluk, yaş ve kuru ağırlık, kuru madde oranı ve su içeriği gibi önemli büyüme parametreleri birlikte değerlendirildiğinde, kökün kuraklık stresinden daha yüksek oranda olumsuz etkilendiği tespit edilmiştir. Bu verilere dayanarak, *L. corniculatus*'un %8 PEG_{6000} uygulaması ile oluşturulan ozmotik potansiyeldeki (-1.03 bar) kuraklık stresi altında yüksek verim kaybına uğrayacağı sonucuna varılabilir.

Anahtar Kelimeler: *Lotus corniculatus* L., Kuraklık stresi, Çimlenme, Fide özellikleri, *in vitro*

1. Introduction

Plants are subject to a variety of biotic and abiotic pressures that may limit their ability to operate to their highest level and jeopardize their survival (Soltanbeigi, 2019; Ahmed et al., 2022). Drought stress is one of the most detrimental abiotic stresses that affect and lower agricultural productivity globally (Yousefi et al., 2020). Cells' equilibrium is impacted by water stress because there is less water available in their cytoplasm. Osmotic and oxidative stress are brought on by a cell's lack of water and harm physiological, biochemical, and molecular functions (Martínez-Santos et al., 2021). The tactics used by plants to deal with water shortages include drought tolerance, drought avoidance, and drought escape (Luo et al., 2020). Potentially, the most important times for water stress in plants are germination and early seedling growth stages (Li et al., 2013). Hence, the understanding of drought impacts on germination and early seedling growth stage is critical for crop production and productivity. Recent studies have examined how plants respond to drought stress in terms of seed germination and seedling growth, particularly those involving agricultural crops (Tong et al., 2021; Ahmed et al., 2022; Beyaz, 2022).

The ability to manipulate the parameters and homogeneity of the culture makes in vitro tissue culture techniques an effective tool for investigating the responses of plants to drought stress (Murshed et al., 2015; Martínez-Santos et al., 2021). PEG is mostly used to extract information from plants relating to drought stress. (Meher et al., 2018). Because PEG cannot penetrate through the plant cell wall and has a higher molecular weight than the other osmotic chemicals utilized (especially PEG_{6000}). As a result, it is frequently utilized in germination and drought research to control osmotic potential (Badr et al., 2020).

In contrast to other common forage legumes like *Medicago sativa* (alfalfa) and/or *Trifolium repens* (white clover), the Lotus genus, native to the Mediterranean basin, is extensively distributed around the world. Its species are suited to many different types of environmental stress and soil conditions. It is well known that *L. corniculatus* L. can grow in soils that are acidic, salinous, low in fertility, and poorly drained (Striker et al., 2005). Compared to other forages, it has been found to improve the output of meat and milk (Hunt et al., 2014). Despite all of these significant traits, the majority of *L. corniculatus* L. is reportedly vulnerable to drought (Bao et al., 2014; Ünlüsoy et al., 2022). However, there are limited reports on the effects of drought on seed germination and early growth stage of *Lotus corniculatus*. Therefore, this study was conducted to examine the germination and early growth stage responses of *Lotus corniculatus* L. to PEG_{6000}-induced drought stress under *in vitro* conditions.

2. Materials and Methods

2.1. Plant Material

In this study, the seeds of the *Lotus corniculatus* L. cultivar ‘AC Langille’, harvested in 2012 and supplied by Utah State University, Plants, Soils and Climate Department, were used as plant material. A cultivar of birdsfoot trefoil (*Lotus corniculatus* L.) called ‘AC Langille’ was created by Agriculture and Agri-Food Canada's Nappan Research Farm. Two cycles of mass selection for winter hardiness and one cycle of mass selection for seedling vigor were used to generate it. The initial material was six unique germplasms chosen from the cultivar ‘Leo’ and made available by the University of Guelph's Crop Science Department (Papadopoulos et al., 1998).

2.2. Plant Tissue Culture and Drought Treatments

The growth medium, standard Murashige and Skoog/Gamborg (Plant Media, USA) (Gamborg et al. 1968), contained 3% sucrose (Research Product International, USA) and 0.25% phytagel (Plant Media, USA). Before autoclaving at 121 ºC, 7.25 psia for 20 minutes, the pH of the medium was set to 5.7. *Lotus corniculatus* seeds were surface sterilized in 50% commercial bleach (Clorox-USA, containing 8.25% sodium hypochlorite) in which 1 drop of Tween-20 (Acros Organics) was added for 20 minutes and then rinsed 3 times with distilled water. For drought stress, seeds were sown on standard MS medium with 4% and 8% w/v PEG_{6000}. According to Michel and Kaufmann (1973), the osmotic potential created by 4% w/v PEG_{6000} is -0.36 bar, and the osmotic potential created by 8% w/v PEG_{6000} is -1.03 bar. Germination of seeds and subsequent seedling growth were carried out at 25±1 °C under white fluorescent lamps at an intensity of 30 μmol m^{-2} s^{-1} (PAR) in a photoperiod of 16 h light and 8 h dark.

2.3. Germination Experiment and Morphological Observations

When the growing radicle lengthened to 2 mm, the seed was considered germinated. Starting on the 9th day, the number of germinations was noted every 24 hours (ISTA, 2003). Mean germination time (MGT) was calculated according to Ellis and Roberts (1980).

$\mathrm{MGT} = \sum \mathrm{Dn}/\sum \mathrm{D}$, where n is the number of freshly germinated seeds on day D, and D is the number of days since the start of the experiment. (Eq. 1)

The percentage of seeds that germinated after being exposed to drought stress were estimated using the equation:

$$\text{Germination percentage (GP)} = (\text{Number of germinating seeds}/\ \text{Total number of seeds}) \times 100$$

(Al-Enezi et al., 2012) (Eq. 2)

Morphological observations (shoot and root length -cm-; fresh and dry weight -mg per plant-) were made on the seedlings that developed 14 days after the start of the experiment (*Figure 1.*). Dry weights were calculated after samples were dried in an oven (VWR Scientific Inc., USA) at 70°C for 48 hours (Beyaz et al., 2011). Water content (WC), dry matter (%) (DM) and vigor index (VI) were calculated according to the following formulas, respectively:

$\text{Water content (WC)} = (\text{fresh weight} - \text{dry weight})/\text{fresh weight} \times 100$ (Zheng et al., 2008) (Eq. 3)

$\text{Dry matter (DM)} = (\text{dry weight}/\text{fresh weight}) \times 100$ (Bres et al., 2022) (Eq. 4)

$$\text{Vigor index (VI)} = (\text{average root length} + \text{average hypocotyl length}) \text{ x germination percentage (GP)}$$

(Abdul-Baki and Anderson, 1973) (Eq. 5)

Germination rate was expressed as the germination rate index (GRI) according to Maguire (1962):

$\mathrm{GRI} = \Sigma\ \text{No of Germinated Seeds}/\Sigma\ \text{No of Days}$ (Eq. 6)

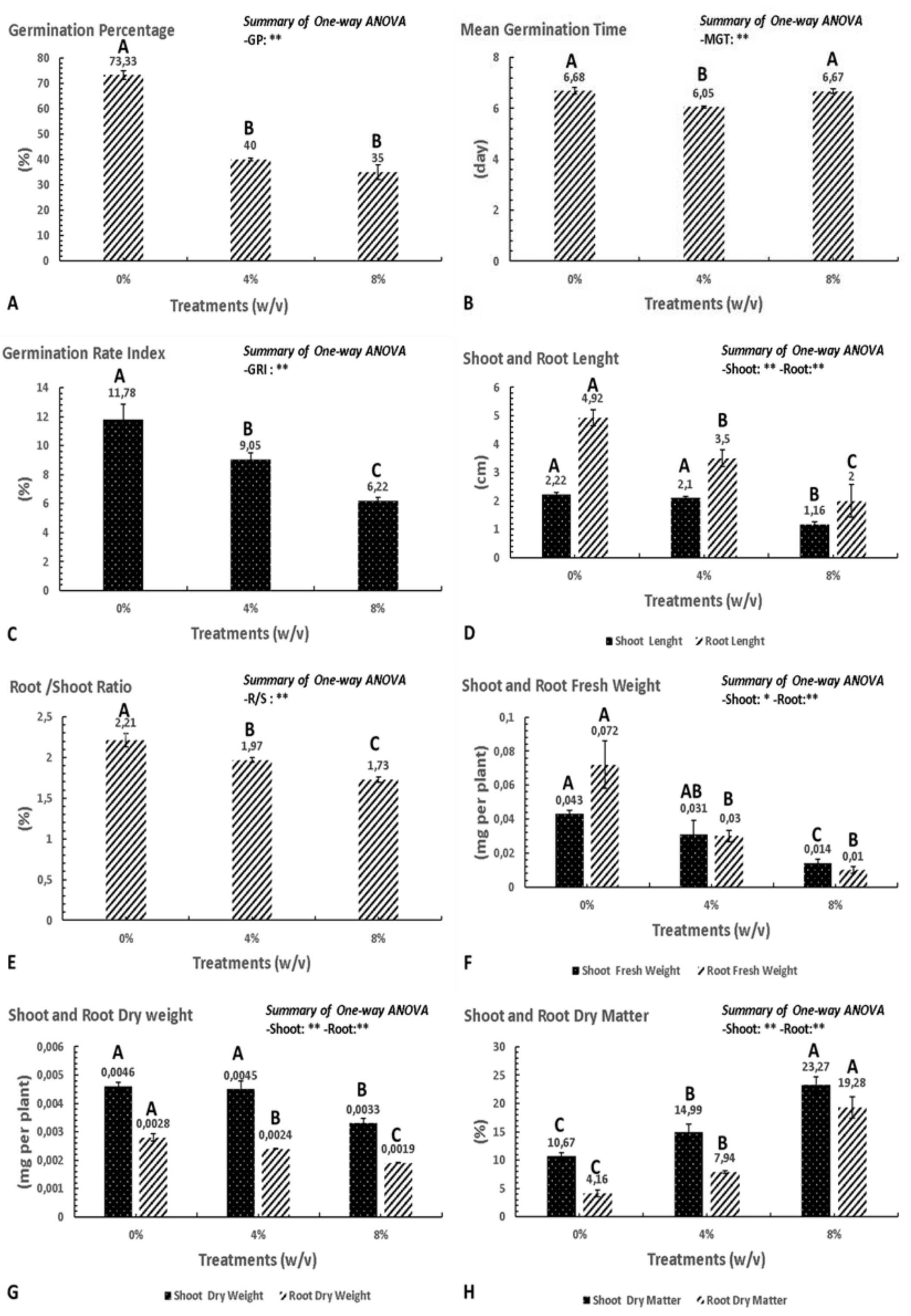
Figure 1. 14-days-old L. corniculatus (cv. 'AC Langille') seedlings at different PEG_{6000} treatments under in vitro conditions. A) 0% PEG_{6000}; B) 4% PEG_{6000}; E) 8% (w/v) PEG_{6000}

2.4. Statistical Analysis

The data collected were analyzed using a one-way Analysis of Variance (ANOVA) in the statistical software package SPSS 22. This analysis aimed to evaluate whether the treatments had a significant impact on the observed parameters. The statistical significance of the means was assessed using the Duncan Multiple Range Test (DMRT) at a significance level of $P<0.05$. Before statistical analysis, the data in percentages were transformed using Arcsine transformation (Snedecor and Cochran 1967).

3. Results and Discussion

Lotus corniculatus cv. AC Langille's germination percentage, mean germination time, and germination rate index (speed of germination) were all significantly ($P<0.01$) impacted by PEG-induced drought stress (*Figure 2A-2B-2C*). The control had the highest percentage of seeds that germinated (73.33%), whereas the 8% PEG_{6000} treatment had the lowest percentage of seeds that germinated (35.00%). The 4% PEG_{6000} treatment had the fastest mean germination time (6.05 days), which was followed by the 8% PEG_{6000} treatment (6.67 days), and the control (6.68 days). The highest germination rate index (speed of germination) value was obtained with 11.78% in the control group, and the lowest value with 6.22% was obtained in 8% PEG_{6000} treatment. Overall, germination percentage was reduced by 45.45% and 52.27% under 4% PEG_{6000} and 8% PEG_{6000} treatments, respectively. The mean germination time was shortened by 9.43% and 0.14% under 4% PEG_{6000} and 8% PEG_{6000} treatment treatments, respectively. As compared to control, germination rate index (speed of germination) was reduced by 23.17% under 4% PEG_{6000}, and 47.19% under 8% PEG_{6000} treatments. The PEG_{6000}-induced drought conditions also had an impact on shoot and root length (*Figure 2D*). Under control, 4% PEG_{6000}, and 8% PEG_{6000} treatments, the shoot length was observed at 2.22cm, 2.10cm, and 1.16cm, respectively. Root lenght was recorded at 4.92cm, 3.50cm and 2cm under control, 4% PEG_{6000} and 8% PEG_{6000} treatments, respectively. The root to shoot ratio recorded 2.21%, 1.97% and 1.73% in control, 4% PEG and 8% PEG treatments, respectively (*Figure 2E*). Shoot lenght was reduced by 5.40% under 4% PEG_{6000} treatment, and 47.74% under 8% PEG_{6000} treatment, in comparison with control. For root lenght, 28.86% and 59.34% reduction was noticed under 4% PEG_{6000} and 8% PEG_{6000}treatments, respectively, as compared with control. The root to shoot ratio was reduced by 10.85% under 4% PEG_{6000} treatment, and 20.81% under 8% PEG_{6000} treatment, in comparison with control. Under conditions of drought brought on by the PEG_{6000} treatments, the shoot fresh weight, root fresh weight, the shoot dry weight, and root dry weight of *L. corniculatus* seedlings was considerably impacted (*Figure 2F-2G*). The control group recorded the highest fresh weight of shoot (0.043 mg) and root (0.072 mg), whereas 8% PEG_{6000} treatment yielded the lowest fresh weight of shoot (0.014 mg) and root (0.010 mg). Similar to this, the control group had the highest dry weights of the shoot (0.0046 mg) and root (0.0028 mg), while 8% PEG_{6000} treatment had the lowest dry weights of the shoot (0.0033 mg) and root (0.0019 mg). The highest reduction in fresh weight of shoot and root by 67.44% and 86.11%, and dry weight of shoot and root by 28.26% and 32.14%, respectively, was seen under 8% PEG_{6000} treatment, on average, compared to control. Shoot and root dry matter ratio was also influenced ($P<0.01$) by the drought treatments (*Figure 2H*). The highest dry matter of shoot (23.27%) and root (19.28%) was obtained in 8% PEG_{6000} while the lowest dry matter of shoot (10.67%) and root (4.16%) was measured under control. Overall, drought stress increased the shoot dry matter ratio by 40.48% and 118.08% while the root dry matter ratio by 90.86 % and 363.46% under 4% PEG_{6000} and 8% PEG_{6000} treatments, respectively. Under conditions of drought brought on by the PEG treatments, the root to shoot dry matter ratio of *L. corniculatus* seedlings was considerably ($P<0.01$) impacted (*Figure 2I*). In terms of root to shoot dry matter ratio, 8% PEG_{6000} had the highest value (0.82%), followed by 4% PEG_{6000} with a value of 0.54% and control with a value of 0.38%. The root to shoot dry matter ratio increased gradually from 42.10% under 4% PEG_{6000} to 118.75% under 8% PEG_{6000} treatment. Water content of shoot and root was also influenced ($P<0.01$) by the drought treatments (*Figure 2J*). The highest water content of shoot (89.32%) and root (95.83%) was obtained in control while the lowest dry matter of shoot (76.72%) and root (80.71%) was measured under 8% PEG_{6000}. When treated with 4% PEG_{6000} and 8% PEG_{6000}, respectively, water contents of shoot and root showed reductions of 4.83% and 3.94% and 14.10% and 15.77%. Similarly, PEG treatment had an impact on *L. corniculatus*'s seedling vigor index (*Figure 2K*). PEG_{6000} treatments caused a significant ($P<0.01$) reduction of seedling vigor index. The highest seedling vigor index value was obtained from the control group with 525.6, followed by 196 with 4% PEG_{6000} treatment and 127.22 with 8% PEG_{6000} treatment, respectively. Under treatments with 4% PEG_{6000} and 8% PEG_{6000}, it dropped by 67.70% and 75.79%, respectively.


Germination Percentage
Summary of One-way ANOVA
-GP: **
(%)
A 73,33
B 40
B 35
0%
4%
8%
Treatments (w/v)
A
Mean Germination Time
Summary of One-way ANOVA
-MGT: **
(day)
A 6,68
B 6,05
A 6,67
Treatments (w/v)
B
Germination Rate Index
Summary of One-way ANOVA
-GRI : **
(%)
A 11,78
B 9,05
C 6,22
Treatments (w/v)
C
Shoot and Root Lenght
Summary of One-way ANOVA
-Shoot: ** -Root:**
(cm)
A 2,22
A 4,92
A 2,1
B 3,5
B 1,16
C 2
Treatments (w/v)
D
Shoot Lenght
Root Lenght
Root /Shoot Ratio
Summary of One-way ANOVA
-R/S : **
(%)
A 2,21
B 1,97
C 1,73
Treatments (w/v)
E
Shoot and Root Fresh Weight
Summary of One-way ANOVA
-Shoot: * -Root:**
(mg per plant)
A 0,043
A 0,072
AB 0,031
B 0,03
C 0,014
B 0,01
Treatments (w/v)
F
Shoot Fresh Weight
Root Fresh Weight
Shoot and Root Dry weight
Summary of One-way ANOVA
-Shoot: ** -Root:**
(mg per plant)
A 0,0046
A 0,0028
A 0,0045
B 0,0024
B 0,0033
C 0,0019
Treatments (w/v)
G
Shoot Dry Weight
Root Dry Weight
Shoot and Root Dry Matter
Summary of One-way ANOVA
-Shoot: ** -Root:**
(%)
C 10,67
C 4,16
B 14,99
B 7,94
A 23,27
A 19,28
Treatments (w/v)
H
Shoot Dry Matter
Root Dry Matter

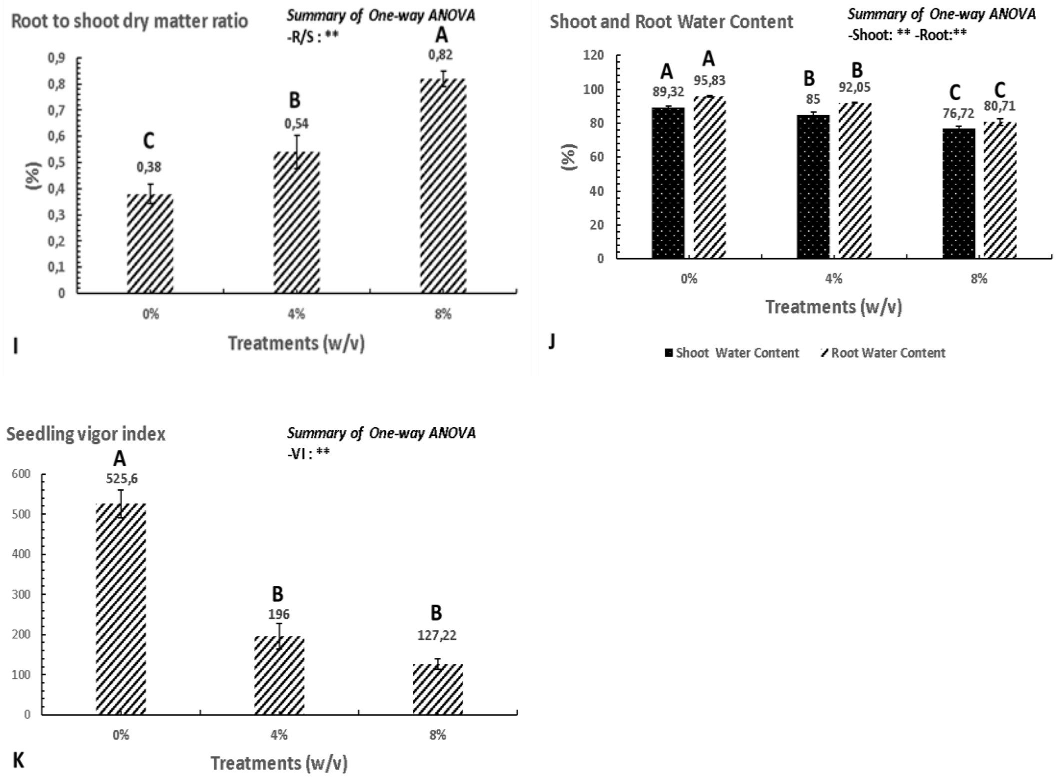


Figure 2. The effect of different levels (0% PEG6000, 4% PEG6000, and 8% PEG6000) of the simulated drought stress on germination percentage, mean germination time, germination rate index, shoot and root length, root shoot length ratio, shoot and root fresh weight, shoot and root dry matter, root shoot dry matter ratio, shoot and root water content, and seedling vigor index of 14-days-old L. corniculatus seedling. **: P<0.01; *: P<0.05.

A significant abiotic factor that significantly reduces crop productivity in the world's arid and semi-arid regions is drought stress. Drought or a water shortage affects germination, which in turn lowers seedling emergence and stops plants from growing further. Successful crop establishment in semi-arid regions mostly depends on good and quick seed germination, which is closely related to seeds' ability to even sprout in the presence of water shortages (Khan et al., 2019). The results of this investigation showed that the growing drought stress steadily hindered (52.27% compared to control with 8% PEG_{6000} treatment) *L. corniculatus* seed germination (*Figure 2A*). Water stress may cause a decrease in the percentage of seeds that germinate because it makes water less permeable through the seed coat and causes seeds to absorb less water initiall (Turk et al., 2004; Bahrami et al., 2012). Similar to the results of this study, it was reported that germination percentage decreased due to increasing drought stress (PEG_{6000}-induced) in other legumes such as sainfoin, alfalfa and white clover (Wang et al., 2009; Hamidi and Safarnejad, 2010; Li et al., 2013; Demiroğlu-Topçu and Özkan, 2016; Bıçakçı et al., 2020; Tong et al., 2021). However, our results highlighted that drought treatments (especially, 4% $PEG6_{000}$) resulted in shortening of mean germination time (*Figure 2B*). Slow germination and slow establishment are a serious problem for *L. corniculatus*. Therefore, researchers try to increase fast of germination of *L. corniculatus* with using some priming techniques. Using PEG_{6000} is one of these priming techniques. Aydınoğlu (2019) reported that the negative effect of drought stress on germination in some forage crops can be reduced by PEG_{6000} priming. However, Jia et al. (2020) noted that a low concentration PEG solution can be employed as an osmotic regulator to control the level and status of water absorption in cells. This can stabilize water absorption in seeds, which will increase their germination and tidy rate. Therefore, it can be interpreted that the concentration of 4% PEG_{6000} treatment makes a positive contribution to the seed germination rate of *L. corniculatus* due to the osmotic potential it creates. However, the mechanism that allows this situation needs to be clarified with more detailed studies (such as physiological, biochemical and molecular). Drought stress influenced the germination rate index (speed of germination) of *L. corniculatus*. The germination rate index (speed of germination) decreased most significantly due to increased PEG_{6000} treatments (*Figure 2C*). As the concentration of the PEG solution increased, the germination of *L.*

corniculatus seeds was also inhibited, according to the study's findings. This could be because low water potential or high osmotic pressure made it difficult for seeds to absorb water during their initial stages of germination, which prevented seeds from germinating normally (Lamia et al., 2012). This show that the negative osmotic effect of PEG on germination, which is in agreement with earlier studies in white clover (Hou and Ma, 2022), sesame (Ahmed et al., 2022), and desert plants (Yousefi et al., 2020). Shoot and root lenght, root shoot ratio, shoot and root fresh weight, shoot and root dry weight parameters are important seedling growth characteristics of seedling establishment. Drought exposure resulted in lower root length, shoot length, root shoot ratio, root and shoot fresh and dry weight at all tested stress levels (*Figure 2D-2E-2F-2G*). Similar findings were made in earlier studies by Ahmed et al. (2022), who found that lower osmotic potential during drought conditions had a negative impact on seedling characteristics of sesame. When lenght, fersh and dry weight were evaluated together for shoot and root organs, it was determined that the highest decrease for lenght and dry weight was in the root under drought stress, however, the highest decrease in fresh weight was in the shoot. It can be interpreted that the further decrease in the lenght and dry weight parameters of the root organ may have occurred because it is the first and harder organ to be exposed to the stress factor. However, the fact that the decrease in fresh weight is less can be interpreted as the fact that the root organ has a higher water content compared to the shoot (*Figure 2J*). Furthermore, the variation in length and fresh and dry weight of shoots and roots could be caused by two factors: (1) impaired cell division and elongation, which eventually slowed down plant growth; and (2) damage from dehydration to the meristem cells of the root and shoot, which interfered with cell division and elongation (Ahmed et al., 2022). A functional balance of growth is provided by the biomass partitioning of roots and shoot growth. Root growth depends on the availability of photosynthates from the shoot, but shoot growth depends on the water absorbed from the root. When under drought stress, stored photosynthates are transferred to the root, allowing it to grow and expand in search of soil water when shoot expansion becomes constrained. The name "Water Spenders" refers to plants that have adapted morphologically to avoid drought stress by increasing root depth, root to shoot ratio, and maintaining water potential above that of the environment (Farooq et al., 2009; Farooq et al., 2012; Bodner et al., 2015; Dissanayake et al., 2019). Therefore, it can be seen as an adaptation developed by this plant so that it can withstand the stress factor more strongly. These findings support earlier research by Hamidi and Safarnejad (2010), Demiroğlu-Topçu and Özkan (2016), Beyaz (2022) and Ahmed et al., (2022) who found significant decreases in growth paramaters of alfalfa, sainfoin, common vetch and sesame under PEG_{6000} induced drought stress. The partitioning of dry matter (DM) in plants is closely correlated with crop productivity during drought stress (Rauf and Sadaqat, 2007). PEG_{6000} induced-drought stress significantly increased dry matter ratio of shoot and root of *L. corniculatus* seedlings (*Figure 2H*). The results revealed that this increase was greater in the root (363.46%) than in the shoot (118.08%) at the highest PEG_{6000} (8%) treatment. However, this increase is indicated by root shoot dry matter ratio (*Figure 2I*). Similar to the research findings, Beyaz (2022) reported that in vitro PEG_{6000} drought treatment (10%) caused an increase in dry matter ratio in both shoot and root organs of common vetch, and this increase was more in the root. A decrease in water content and disruption to cellular membranes, which aid the body in coping with abiotic stresses such as drought, are the primary impacts of stress (Zhou et al., 2012). According to the results of the research, drought treatments caused a decrease in both shoot and root organ water content (*Figure 2J*). This decrease was greater at the root (15.77%) under highest PEG treatment, in comparison with control. Meher et al. (2018) stated that with an increase in PEG_{6000} concentrations (5%, 10%, 15%, and 20%), relative water content (RWC) dramatically decreased in both leaves and roots of peanut. Seed vigor, a crucial indicator of seed quality, assesses the likelihood of rapid and uniform plant emergence (Yousef et al., 2020). Drought stress has an inhibiting influence on the seedling vigor index in the current investigation (*Figure 2K*). In this study, the calculation of seedling vigor index was made by considering seed germination, shoot and root length. Therefore, the sharp decrease in these parameters caused by drought stress also affected seedling vigor index. A very significant loss of 75.79% occurred in seedling vigor index of *L. corniculatus* cultivar AC Langille with 8% PEG_{6000} treatment. The findings of present study are in agreement with the results of Ahmed et al. (2022), Badr et al. (2020), Yousef et al. (2020), and Hou and Ma (2022) who reported the PEG_{6000}-induced drought stress caused a decrease in seedling vigor index.

4. Conclusions

In nutshell, PEG_{6000} induced-drought stress significantly inhibited germination and seedling growth of *Lotus corniculatus* cultivar ˈAC Langilleˈ. When seed germination parameters (germination percentage+germination rate index) were evaluated cumulatively and their means were calculated, 8% PEG_{6000} treatment caused a 49.73% decrease. On the other hand, in the 4% PEG_{6000} treatment, a 9.43% shortening of the mean germination time occurred, most likely due to the low dose effect. When the above-ground parameters (lenght+fresh and dry weight+ water content of shoot) were evaluated cumulatively and their means were calculated, 8% PEG_{6000} treatment caused a decrease of 37.69%. However, when the below-ground parameters (lenght+fresh and dry weight+water content of root) were evaluated cumulatively and their means were calculated, 8% PEG_{6000} treatment caused a decrease of 47.37%. On the other hand, the above-ground dry matter ratio increased by 118.08% and the below-ground dry matter ratio increased by 363.46%. Additionally, *L. corniculatus* has lost 75.79% of its seedling vigor index under 8% PEG_{6000} treatment. Therefore, in the light of these data, it is seen that the *L. corniculatus* will suffer a high yield loss under the drought stress at the osmotic potential (-1.03 bar) created by 8% PEG_{6000} treatment. In future studies, it is recommended that the germination and initial seedling growth stage of salt stress, which is defined as a sister to drought stress in nature, should be tested to determine the *L. corniculatus* responses, which are stated to be sensitive to salinity.

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