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Research
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Researching germination properties of bean (*Phaseolus vulgaris* L.) under polyethylene glycol osmotic stress and saline conditions*

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Abstract

Plant growth is negatively affected by environmental factors such as salinity and drought. This research was carried out in the laboratory to determine the germination characteristics of bean cultivar called "Akkuş" under drought and salt stress conditions. The experiment was set up using a completely randomized experimental design with ten replications. For the purpose of the study, germination rate (%), radicle length (cm), plumule length (cm), hypocotyl length (cm), radicle fresh weight (g), plumule fresh weight (g), and hypocotyl fresh weight (g) were calculated. Polyethylene glycol (PEG) 6000 was used to create a dry environment and NaCI was used for a saline environment. Different doses of NaCl (0 (control), 50, 100, 150, and 200 mM) and different osmotic potential levels of PEG 6000 (0 (control), -0.2, -0.4, -0.6, and -0.8 bar) were used in the experiment. The germination rate of bean decreased with increasing salt concentrations. Similarly, an increase in PEG 6000 osmotic potential levels caused a decrease in the parameters. The highest values were obtained in the control groups. As a result of the study, increase in salinity and drought stress cause negative effects on bean germination properties.

Key words: Bean, Drought, Germination, Polyethylene glycol, Salinity

Polietilen glikol osmoz stresi ve tuzlu koşullar altındaki fasulye (*Phaseolus vulgaris* L.) bitkisinin çimlenme özelliklerinin araştırılması

Öz

Bitki büyümesi tuzluluk ve kuraklık gibi çevresel faktörlerden olumsuz etkilenmektedir. Bu araştırma kurak ve tuzlu stres koşulları altında Akkuş fasulye çeşidinin çimlenme özelliklerinin belirlenmesi amacıyla laboratuvarda yürütülmüştür. Çalışma tesadüf parselleri deneme desenine göre 10 tekrarlı olarak yürütülmüştür. Çalışmada çimlenme oranı (%), radikula uzunluğu (cm), plumula uzunluğu (cm), hipokotil uzunluğu (cm), radikula yaş ağırlığı (g), plumula yaş ağırlığı (g) ve hipokotil yaş ağırlığı (g) değerleri ölçülmüştür. Kurak ortam oluşturmak amacıyla Polietilen glikol (PEG) 6000, tuzlu ortam oluşturmak amacıyla da NaCI kullanılmıştır. Denemede farklı dozlarda NaCl (0 (kontrol), 50, 100, 150, 200 mM) ve farklı osmotik potansiyel seviyelerinde PEG 6000 (0 (kontrol), -0.2, -0.4, -0.6, -0.8 bar) uygulanmıştır. Artan tuz konsantrasyonları karşısında fasulye tohumlarında çimlenme oranı düşüş göstermiştir. Benzer şekilde PEG 6000 uygulamalarında da dozlar arttıkça incelenen parametrelerde düşüşler meydana gelmiştir. En yüksek değerler kontrol gruplarında elde edilmiştir. Çalışma sonucunda tuzluluk ve kuraklık stresinin artmasıyla birlikte fasulye bitkisinde çimlenme özelliklerinde olumsuz etkiler meydana geldiği saptanmıştır.

Anahtar kelimeler: Çimlenme, Fasulye, Kuraklık, Polietilen glikol, Tuzluluk

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Introduction

Stress factors are environmental factors that negatively affect plant growth. A wide variety of biotic and abiotic environmental factors in nature cause stress in plants. Factors such as drought and salinity are called abiotic stress factors (Çolak et al., 2018).

Salt stress is one of the major abiotic stress factors affecting plant growth, especially in arid and semi-arid regions. Salt stress causes osmotic and ionic stress in plants, thereby affecting plant growth and development and these negative effects of salt stress vary depending on the type of salt, the level and duration of stress, and the growth phase of the stressed plant (Çulha and Çakırlar, 2011). The harmful effects of salt may arise from the occurrence of ion toxicity, increased osmotic pressure, and decreased water availability (Yılmaz et al., 2011). Although salt stress affects all development phases of plants, the most sensitive period has been reported to be germination (Khan et al., 2009). Salinity stress mostly reduces the germination percentage and delays the onset of germination (Bojovic et al., 2010).

Drought is regarded as the second major abiotic environmental stress. Drought is caused by a decline in rainfall, leading to a severe decline in production in turn (Ghiyaasi and Tajbakhsh, 2013). Drought stress generally arises from the reduction of usable water in the soil or the change in atmospheric conditions through sweating and evaporation. Depending upon plant species, certain stages such as germination, seedling or flowering could be the most critical stages for water stress (Ahmad et al., 2009).

Even if the plant varieties belong to the same species, they may differ in their drought tolerance. Some plant varieties may be drought-tolerant and thus continue to grow, develop and be fertile in dry environments, while other drought-sensitive varieties of the same species may suffer damage that might cause severe yield losses even in small amounts of water loss. Many researchers have noted that drought stress might be simulated in plants using PEG in vitro and drought-tolerant genotypes might be selected (Chen et al., 2010; Zhu et al., 2005). PEG is a macromolecular osmotic agent, cannot penetrate the seed and has no toxic effect. Because PEG does not infiltrate the apoplast, it affects the osmotic pressure and causes the withdrawal of water, thereby leading to drought stress (Petrovic et al., 2016). Salinity acts like drought on plants, preventing roots from performing their osmotic activity where water and nutrients move from an area of high concentration (Bojovic et al., 2010). Beans (*Phaseolus vulgaris* L.) are edible legume plants that have the most cultivation area and production in the world and also very important for human nutrition due to its high protein level. Bean is a plant that might have different reactions to salinity and drought depending on its varieties. Germination and seedling characteristics are the most viable criteria used for selecting stress tolerance in plants and germination percentage and seedling growth are most important for cultivar selection (Moradi and Zavareh, 2013). In this study, it was aimed to determine the effects of salt stress and drought stress on germination and seedling development in bean (*Phaseolus vulgaris* L.).

Materials and Methods

The experiment was carried out under in vitro controlled condition in the laboratory of the Department of Field Crops in the Faculty of Agriculture of Ordu University in 2019. In the research bean cultivar called Akkuş were used. During the experiment, different salt concentrations (0 (control), 50, 100, 150, 200 mM) and different osmotic potential levels of PEG 6000 (0 (control), -0.2, -0.4, -0.6, -0.8 bar) were dissolved with pure water in 500 ml sterile jars. PEG 6000 was used to simulate a dry environment and osmotic pressure was created on equation supplied by Michel and Kaufman method (Michel and Kaufman, 1973).

$$\psi_s = -(1.18 \times 10^{-2})\, C - (1.18 \times 10^{-4})\, C + (2.67 \times 10^{-4})\, CT + (8.39 \times 10^{-7})\, C^2T$$

ψ_s= osmotic potential (bar), C= amount of polyethylene glycol (g/l), T= temperature (°C)

Sodium chloride (NaCI) was used to create a saline environment. Before germination, the seeds were kept in 5% sodium hypochlorite for 5 minutes and then washed with pure water. Later, the seeds were placed in Petri dishes that were 11 cm in diameter and covered with a double layer of filter paper. Ten seeds were placed in each Petri dish. 10 ml of the prepared solutions was added to the seeds (Keshtiban, 2015; Mena et al., 2015) and the dishes were closed by parafilms and left to germinate in completely dark and 25 ±1°C in the incubator. Seeds with a radicle length of 2 mm were considered germinated. At the end of the seventh day, the count was completed. Germination rate (%), radicle length (cm), plumule length (cm), hypocotyl length (cm), radicle fresh weight (g), plumule fresh weight (g), and hypocotyl fresh weight (g) were calculated.

Germination rate (%) = (Number of germinating seeds / total number of seeds) ×100

The bean seed consists of two cotyledons which are attached to one end of the hypocotyl, and the plumule which grows out from the epicotyl between the two cotyledons (Kater, 1927). The radicle–hypocotyl junction was identified by the color of the germination shoot (white color part was considered as radicle, the other color part as hypocotyl) (Zhu et al., 2006). The experiment was set up using a completely randomized experimental design with ten replications. All data were analysed statistically by JMP statistical software version 11. The averages found statistically significant in the analysis of the data were grouped according to the "LSD Multiple Comparison Test". Variance analysis was evaluated according to P<0.05 significance level.

Result and Discussion

In the study, it was determined that salt and drought stress on bean seeds had significant effects on all of the parameters examined. The effects of NaCl and PEG on the total germination rate of bean seeds were statistically significant (P<0.01). The germination rate, which was 92.8% in the control group decreased with increasing NaCl doses. The lowest germination rate was achieved at the highest salt dose of 200 mM (Figure 1). The germination rate decreased from control to 80%, 47%, 25% and 19% at 50, 100, 150 and 200 mM salt doses, respectively. In a study, Kaymakanova (2014) examined the effects of stress caused by 100 mM dose of NaCl on the bean cultivars and reported that created salt stress decreased the germination rate in all bean cultivars compared to the control. Mena et al., (2015) used NaCl as selected agent to induce salt stress and tried to determine the effects on the germination of bean. As a result of the study, differently from our results there was no difference in the germination rate of 50, 100 and 150 mM NaCl doses compared to the control, while there was a 50% decrease in the 200 mM dose, and no germination occurred in the 250 mM dose. Different cultivars of plants react differently to salinity, and this is due to genetic variability between them (Asmare, 2013). The decrease in germination rate due to the increasing salt doses, together with the toxicity of Na and Cl ions, is caused by the rising osmotic pressure preventing the water required for germination from entering the seed (Ekmekçi et al., 2005).

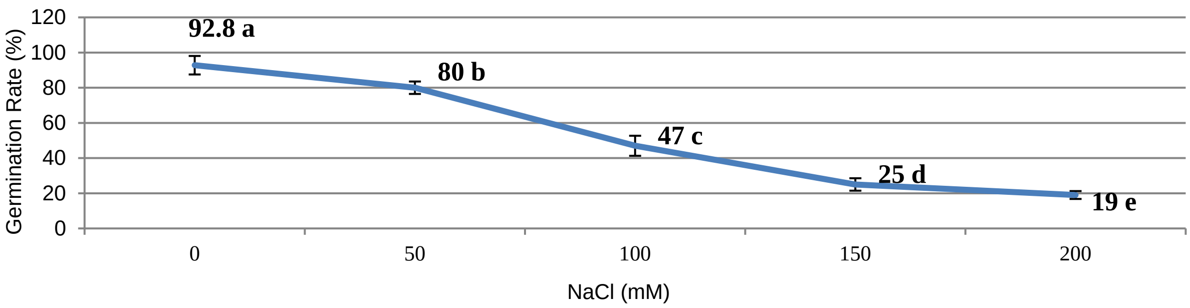

Figure 1. Effects of salt doses on germination rate (%)

Similarly to the effect of salt stress under drought stress germination rate decreased with increasing PEG solution (Figure 2). The lowest germination rate was observed in the highest PEG solution (-0.8 bar) with 28%. Gholami et al., (2009) were examined effects of different PEG solutions on the germination of the beans and as a result germination rate decreased progressively as osmotic potential increased to -0.2 bar, and in drought stress greater than -0.4 bar germination rate remained nearly constant. Petrovic et al. (2016), reported that the germination rate was 97% in the control cultivars on peas, while it was 83% in -0.2 bar PEG solution and 79% in -0.3 bar PEG solution. Drought stress reduces protein synthesis of seeds, slows cell division and ultimately reduces germination power (Bray, 1997). It is thought that these mentioned effects of salinity and drought reduce germination in our study. The effects of both NaCl and PEG on the bean radicle length were found to be statistically significant (P<0.01). Radicle length was measured as 5.304 cm in the highest in control group and decreased as stress doses increased. The reduction in the 50 mM NaCl dose compared to the control was 23%, while in the 100 mM NaCl dose this ratio was 28%. At 150 and 200 mM NaCl doses, this decrease occurred around 99% (Figure 3). In PEG osmotic potential levels, from control to -0.2 and -0.4 bar potential levels the radicle length decreased 17% and 29%, respectively. This decrease is quite high at -0.6 and -0.8 bar and is around 99%.

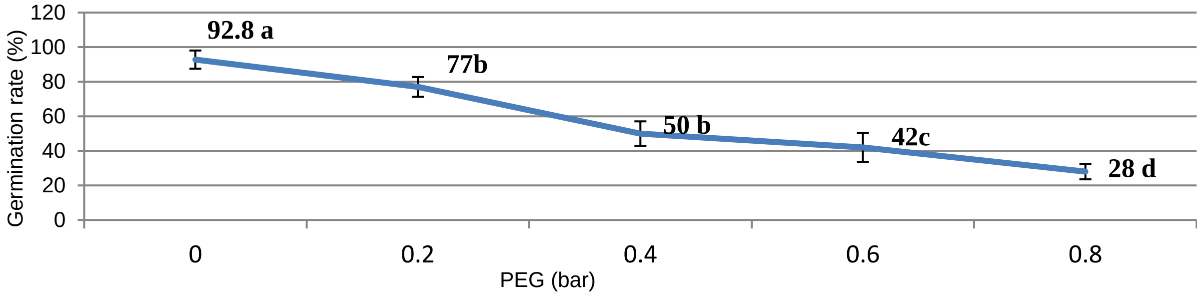

Figure 2. Effects of osmotic potential levels on germination rate (%)

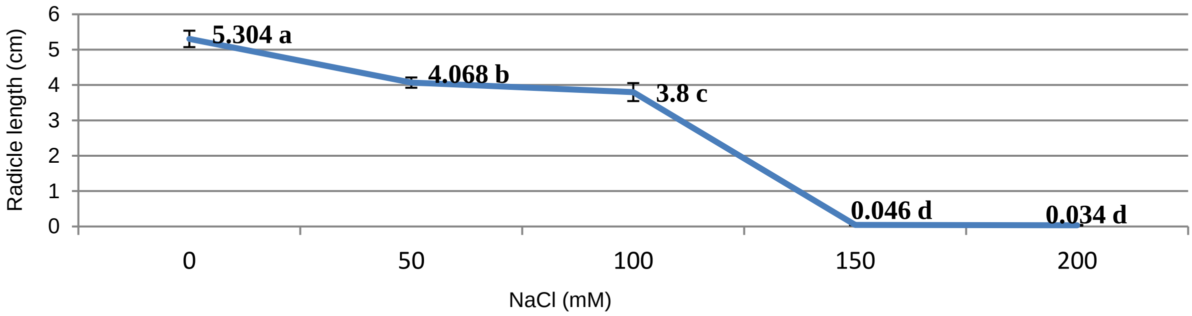

Figure 3. Effects of salt doses on the radicle length (cm)

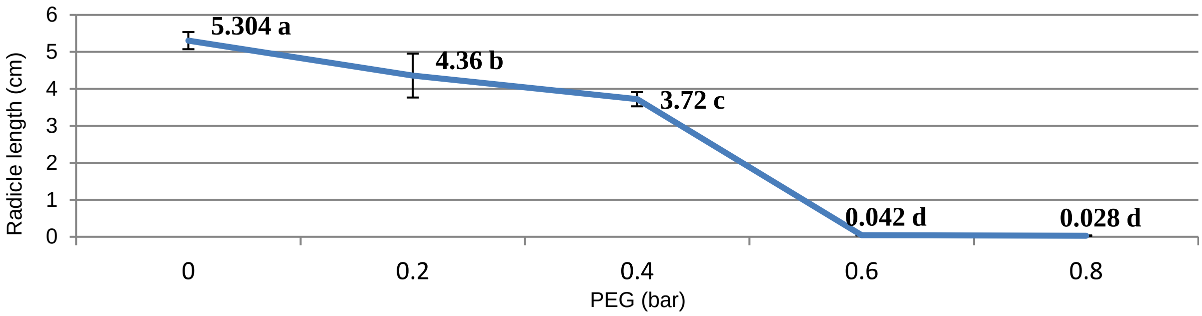

Figure 4. Effects of osmotic potential levels on the radicle length (cm)

Plumule length was influenced by salt and drought stress and showed similar decreasing like radicle length and had significant differences with control. It was measured 5.904 cm in the control group. In particular, there was a 99% decrease compared to the control at 150 and 200 mM doses of NaCl, and -0.6 and -0.8 bar PEG (Figure 5 and Figure 6).

The effects of NaCl and PEG on the hypocotyl length were statistically significant ($P<0.01$). There were decreases in the hypocotyl length with the increased stress level. In contrast, 150 and 200 mM NaCl doses and -0.6 and -0.8 bar PEG cause a 99% reduction in radicle and plumule length compared to control, while the reduction rate in hypocotyl length compared to control is 48% and 63%, 62% and 69% because of these doses (Figures 7 and 8). Mena et al. (2015), used different salt concentrations on beans and reported that germinate of plants at 200, 250 and 300 mM NaCl doses were drastically affected and seeds that did not germinate produce no radicle. Abdel-Haleem and El-Shaieny (2015), reported in his study that the length of radicle and plumule decreased with increasing salt doses in cowpea compared to control. Nezamy et al., (2010) study drought stress on lentil and showed that increasing osmotic potential levels the radicle and plumule length decreased, the highest values were obtained in control groups. In another study with 3 leguminous species under salt and druogh stress, increasing the PEG resulted in a decrease in the hypocotyl length, but no significant difference between 5% PEG and the control was observed and the hypocotyl length was noticeably inhibited by 100 and 150 mM NaCl but not by 50 mM (Wu et al., 2011).

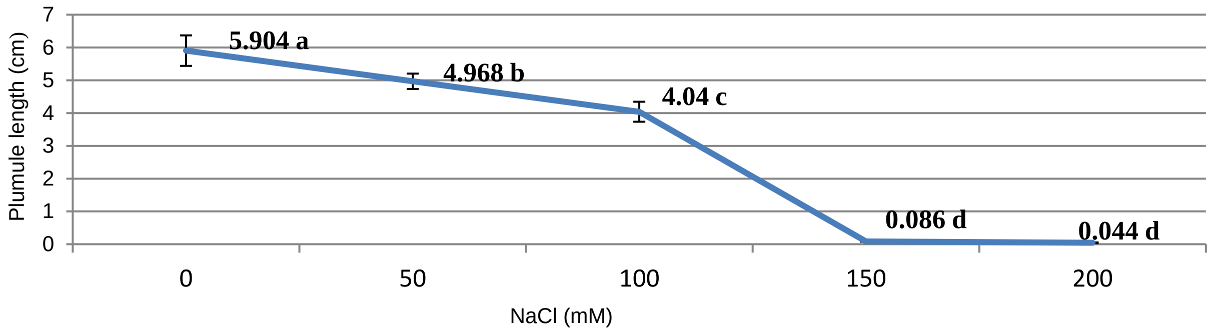

Figure 5. Effects of salt doses on the plumule length (cm)

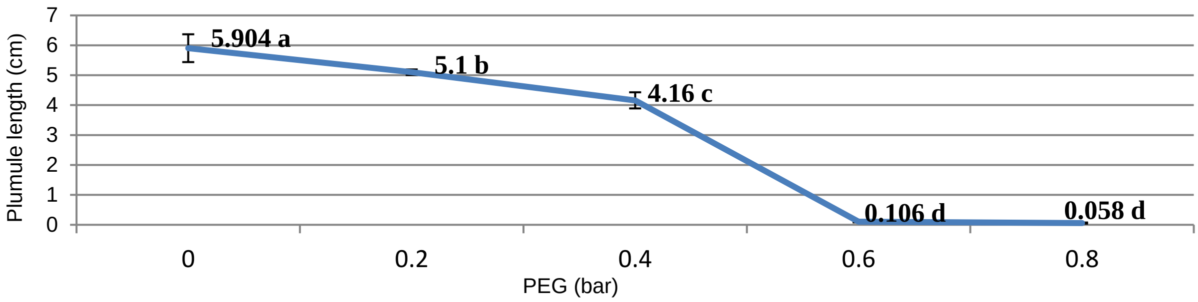

Figure 6. Effects of osmotic potential levels on the plumule length (cm)

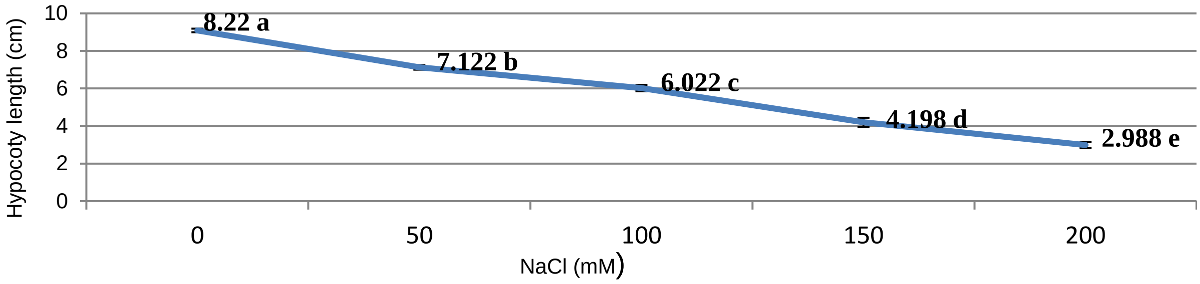

Figure 7. Effects of salt doses on the hypocotyl length (cm)

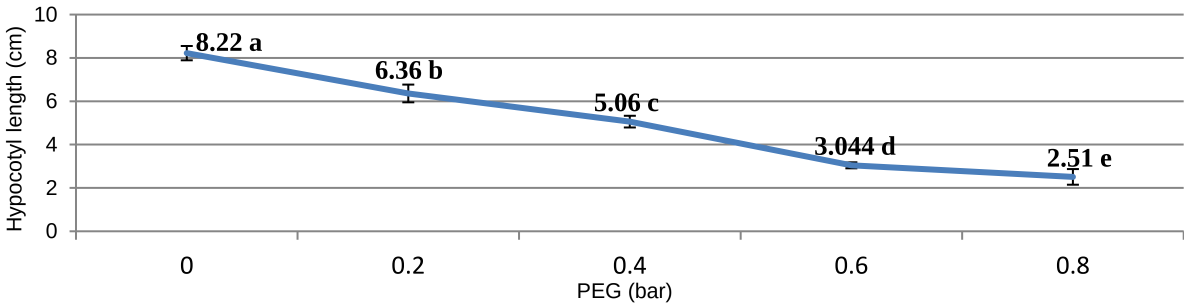

Figure 8. Effects of osmotic potential levels on the hypocotyl length (cm)

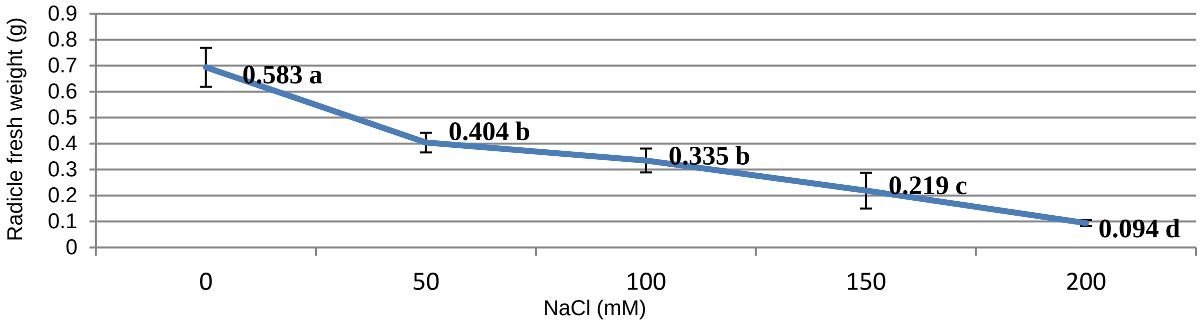

Figure 9. Effects of salt doses on radicle fresh weight (g)

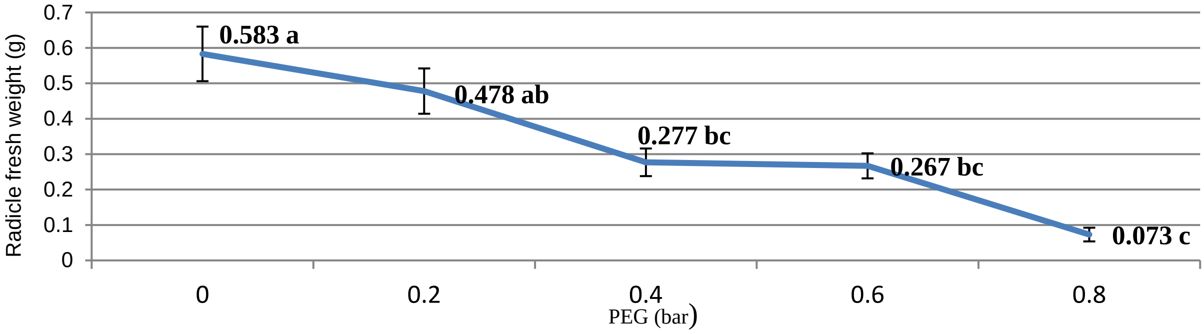

Figure 10. Effects of osmotic potential levels on radicle fresh weight (g)

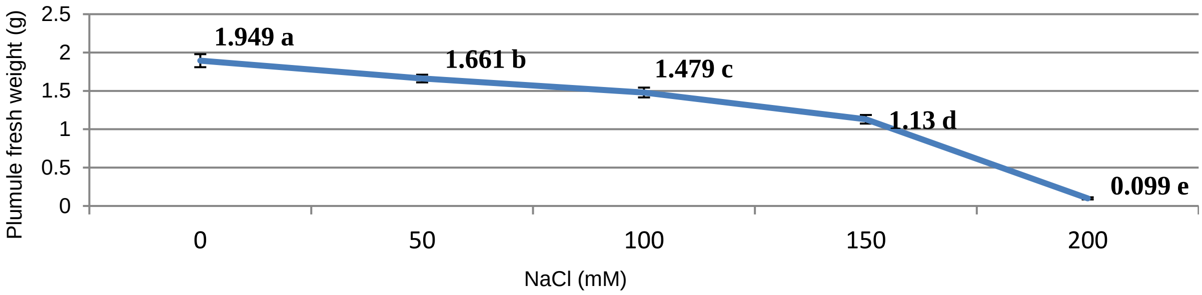

Figure 11. Effects of salt doses on plumule fresh weight (g)

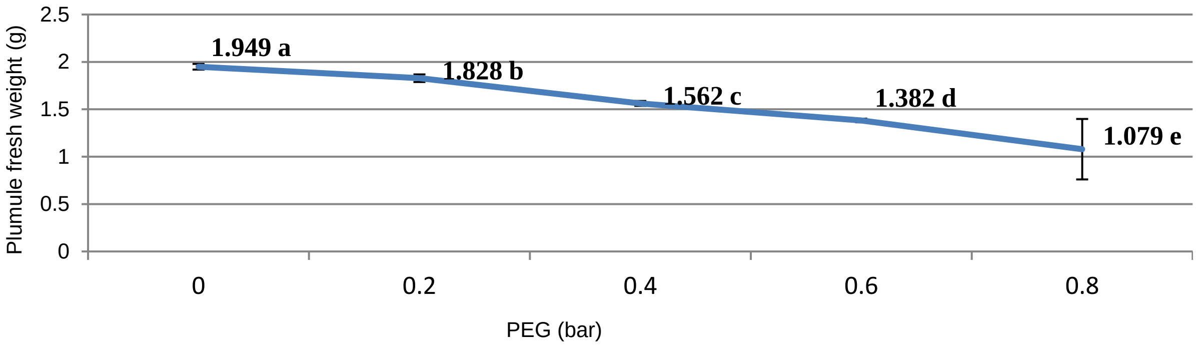

Figure 12. effects of osmotic potential levels on plumule fresh weight (g)

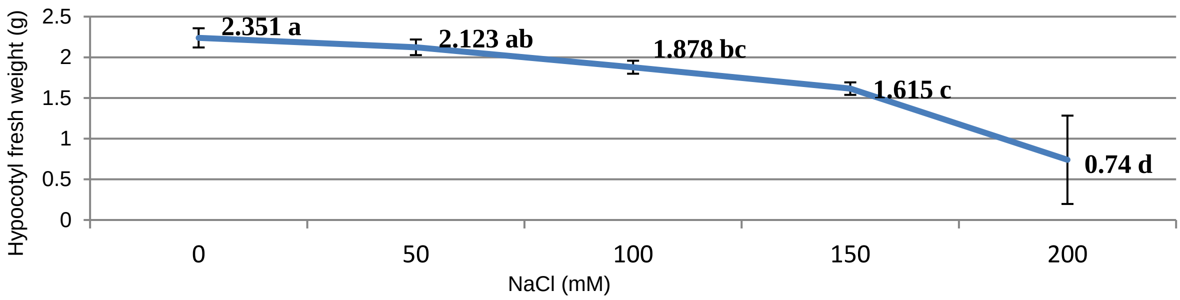

Figure 13. Effects of salt doses on hypocotyl fresh weight (g)

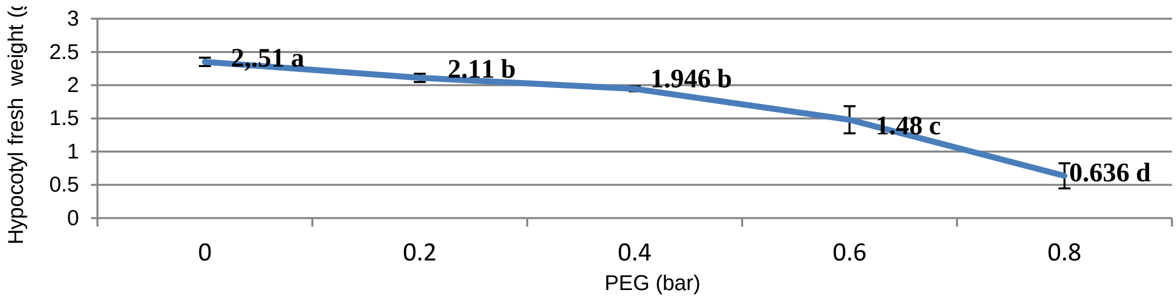

Figure 14. Effects of osmotic potential levels on hypocotyl fresh weight (g)

The fresh weights show that growth was inhibited by NaCl and PEG. The effect of salt doses on the radicle fresh weight was significant at 1% while the effect of PEG was significant at 5% (Figures 9 and 10). The plumule fresh weight was 1.949 g in the control group and decreased as the NaCl and PEG increased. The effect of both stress conditions on the plumule fresh weight was significant at 1% (Figures 11 and 12). In hypocotyl fresh weight, the most severe effects were observed at the highest dose of NaCl and PEG with 200 mM salt and -0.8 bar, the most significant decreases occurred at these stresses. The effect of the treatments on the hypocotyl fresh weight was significant at 1% under both stress conditions (Figures 13 and 14). Petrovic et al. (2016), created NaCl (50, 100, and 150 mM) and PEG (-0.2 and -0.3 bar) on pea and reported that increased concentrations of both NaCl and PEG resulted in a decrease in the radicle, plumule and hypocotyl fresh weights. Murillo-Amador et al. (2002), used PEG-8000 solutions (0, -0.2, -0.4, -0.6, and -0.8 bar) on cowpea and found a decrease in the radicle, plumule and hypocotyl fresh weights at increasing stress levels, while the greatest decrease was at the -0.8 bar PEG osmotic level. Khadri et al., (2007) reported that in common bean cultivars, shoot and root weights were negatively affected by the salt stress. The results are similar to the mentioned studies. In many crop plants, early seedling growth and seed germination are most sensitive stage to environmental stress (Abdel-Haleem and El-Shaieny, 2015). Reducing in water absorption by seed because of drought stress reduced secretion of enzyme activity and it has negatively effect on seedling growth (Kafi et al., 2005). Salt and drought stress lead to decreased water availability promote the synthesis of abscisic acid and it causes negative effect on seedling growth (Cutler et al., 2010). In our study, it is thought that the decreases in the seedling growth as a result of drought and salt stress are due to the reasons mentioned above.

Conclusion

As a result of this study it was determined that the salt and drought stress negatively affect the germination and seedling development of the bean plant. The analysis results showed that all stress level; both salt and drought, had significant effects on all of the properties examined. Especially 150 mM and 200 mM NaCl doses and -0.6 bar and -0.8 bar PEG 6000 osmotic levels caused more decrease compared to the control than other doses.

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