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Research Article

Investigation of Germination and Early Seedling Development of Some Flax (*Linum usitatissimum* L.) Seeds Under Salt Stress

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ABSTRACT

Salinity, one of the abiotic stress factors, causes various physiological damages in plants. Salt stress significantly affects plant growth and development. One of the ways to reduce the negative effect of salt stress, which has a limiting effect on plant growth, is to determine the tolerance level of plants. This study was carried out under controlled conditions in order to examine the effect of salt stress on the germination of flax plant, which is one of the important industrial plants with various usage areas around the world. In this study, 3 flax varieties (Somme, Midin and Norman) and 4 salt concentrations (control, 50 mM, 100 mM and 200 mM) were used. In this experiment, germination rate, radicle length, plumula length, seedling length and seedling fresh weight characteristics of seeds under salt stress were examined. Statistically significant differences were detected between all the properties examined in the study, both among the cultivars and the salt doses. The study revealed that the control (pure water) group obtained the highest values in all the examined properties, but these values significantly decreased as the salinity severity increased. It was determined that the 200 mM salt dose significantly affected the germination of flax. 'Somme' flax variety was the most tolerant variety against salt doses in terms of the properties examined.

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Tuz Stresine Maruz Bırakılan Bazı Keten (*Linum usitatissimum* L.) Tohumlarının Çimlenme ve Erken Fide Gelişiminin Araştırılması

ÖZET

Abiyotik stres faktörlerinden biri olan tuzluluk, bitkilerde birçok fizyolojik hasarlara yol açmaktadır. Tuz stresi bitki büyüme ve gelişmesinde önemli bir rol oynar. Bitki gelişimini sınırlayıcı etkiye sahip olan tuz stresinin olumsuz etkisini azaltmanın yollarından biri de bitkilerin tolerans düzeyinin belirlenmesidir. Bu çalışma, dünyada birçok kullanım alanına sahip önemli endüstri bitkilerinden biri olan keten bitkisinin tuz stresi altında çimlenme ve fide gelişimi üzerine olan etkisini incelemek amacıyla kontrollü şartlar altında yürütülmüştür. Çalışmada 3 adet keten çeşidi (Somme, Midin ve Norman) ve 4 adet tuz konsatrasyonu (kontrol, 50 mM, 100 mM ve 200 mM) kullanılmıştır. Araştırmada tuz stresine maruz bırakılan tohumların çimlenme oranı, radikula uzunluğu, plumula uzunluğu, fide boyu ve fide yaş ağırlığı parametreleri incelenmiştir. Araştırmada incelenen tüm özelliklerde hem çeşitler hem de tuz dozları arasında istatistiksel olarak önemli farklılıklar tespit edilmiştir. Çalışma sonucunda incelenen tüm özelliklerde en yüksek değerler kontrol (saf su) grubundan elde edilirken, tuzluluk şiddeti arttıkça bütün değerlerde düşüşler meydana gelmiştir. Ayrıca, 200 mM tuzluluk dozunun keten çimlenmesini önemli ölçüde etkilediği çalışmanın sonuçları arasında yer almaktadır. İncelenen özellikler açısından 'Somme' keten çeşidi tuz dozlarına en toleranslı çeşit olmuştur.

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INTRODUCTION

Flax (*Linum usitatissimum* L. 2n=30) is one of the self-pollinated oilseed plants that complete the vegetation period in one year. While oil is obtained from the seeds of flax, fiber is obtained from the stem of some forms. Flax is one of the oldest plants cultivated for its fiber as well as its oilseed (Dubey et al. 2020). Flax seeds contain approximately 40-45% oil and this oil is used in various industrial fields such as making paints, soaps, varnishes, and printer ink (Cloutier et al. 2009). The oil obtained from flax seeds is widely used in the paint and flooring industry, as it dries faster than other vegetable oils (Bayrak et al. 2010). Flax oil has also been recognized as an excellent source of micronutrients. It is also known to be rich in dietary fiber, protein, vitamin B1, lignin and linoleic acids, which are essential fatty acids (Kajla et al. 2015; Goyal et al. 2016). Although flax, which is not very selective in terms of soil properties, can be grown easily in most soil types, it is adversely affected by heavy textured soils with poor drainage. The plant can easily grow in soils with a pH between 6 and 6.5. The vegetation period of flax is short. Although the vegetation period varies according to the variety and the geographical conditions in which it is grown, it completes its development in an average of 90 to 120 days. Since the life cycle of the plant is short, it ensures that two crops are taken from the same field in one year. Therefore, it can be considered as a good rotation plant. The adaptation of flax to different climatic areas is high. The climate requirements of flax differ according to the purpose of cultivation (for fiber or seed purposes). Particularly subtropical areas are more suitable for the seed production of flax, which is widely distributed in humid areas with a mid-belt climate. Humid climatic regions are important for flax cultivation, and especially cool, frequent rainy, coastal areas (in fiber-purpose cultivation) are very ideal.

Salinity stress, to which plants are exposed, causes an increase in osmotic pressure and a deterioration of the balance in ion uptake (Tahjib-Ul-Arif et al. 2018). Plants are adversely affected by salinity stress at all stages of growth. Response to salinity stress varies not only between plant species but also between different varieties of the same species (Bojović et al. 2010). Wang et al. (2009) reported that many plant species are more sensitive to salinity during the early stages of growth, such as germination and seedling formation stages. The severity of salt stress and the resistance of the plant to salinity are first determined during the germination and seedling growth stages, because the rapid and uniform emergence of seedlings is an important prerequisite for obtaining efficient and high quality crops (Sadeghi and Robati 2015). Misra and Dwivedi (2004) reported that the germination phase is the most critical growth period in plants and that plants are greatly affected by salinity during this phase.

In this study, the resistance of flax, an important alternative oil plant, to salt stress during germination and early seedling growth stages was investigated.

MATERIAL AND METHODS

This study was carried out under controlled conditions to determine the effects of salt doses applied to three different flax varieties (Norman, Somme, Midin) on the germination and early seedling development stages of seeds. The study was carried out in three replications according to the factorial experimental design in random plots. In the study, double-layered Whatman paper was placed in sterilized petri dishes for each application and 30 flax seeds sterilized in a 10% NaCl solution were placed in each petri dish. Petri dishes were left open for a while to dry the seeds placed in 11 cm diameter petri dishes. After the seeds dried, 7 mL of the prepared salt concentrations were added to them (Moghaddam et al. 2018). The same amount of distilled water was added to the control group. The salinity concentrations were prepared as 0 (control group), 50, 100 and 200 mM, which were determined based on previous studies (Moghaddam et al. 2018). Petri dishes were wrapped with parafilm to prevent moisture loss due to evaporation. The prepared petri dishes were left to germinate in the climate cabinet at 25 °C at 70% relative humidity and the germinated seeds were counted at the same time every day (every 24 hours) for 7 days (Bilgili et al. 2011). In the experiment, seeds with a radicle of 2 mm were considered germinated (Mostafavi 2012). In the study, radicle length, plumula length, seedling length seedling fresh weight and germination rate were measured. The germination rate was calculated by dividing the the number of germinated seeds by the total number of seed then multiplying them by 100 (Maquire 1962). For seedling lengths, radicle and plumule lengths were measured separately, and then the seedling length was determined by adding both lengths (ISTA 1984). The radicle and plumule were weighed and the fresh weights of the seedlings were determined. The data obtained from the study and variance analyses were performed using the JMP statistical package program, and the differences between the means and multiple comparisons were calculated according to the LSD test.

RESULTS AND DISCUSSION

In this study, the effects of different salt concentrations on the germination and seedling development periods of some flax (*Linum usitatissimum* L.) cultivars were investigated. In the study, it was observed that there were significant differences in terms of radicle length, plumula length, seedling lenght, seedling fresh weight and germination rate (P<0.05).

Significant differences between cultivars and doses in terms of radicle length can be seen in Table 1. From the table, it is seen that flax seeds belonging to different varieties are adversely affected by increasing salt stress, and radicle length decreases as the severity of salinity increases. While the maximum radicle length was obtained from the control group, the lowest average value was obtained from the highest salt concentration of 200 mM. While the Somme variety had a radicle length of 91.30 mm in the control group, this value decreased as the intensity of the doses increased. Norman variety was the most affected variety in terms of radicle development at 200 mM salt concentration. Since plant roots are in direct contact with the soil, they take water and nutrients from the soil and transmit them to the upper parts of the plant. Therefore, root development is one of the most important parameters in terms of salt stress (Moghaddam et al. 2018). Jamil et al. (2006) reported that root and shoot lengths provide important clues in the response of plants to salt stress. Moghaddam et al. (2018) investigated the responses of some flax varieties to salt stress. In their study, they stated that the best development in terms of radicular length was obtained from the control group and that radicula development slowed down as the severity of salinity increased. Abido and Zsombik (2019) reported that the length of the radicle was changed at different salt doses and that the radicular length clearly decreased as the salt intensity increased.

In terms of plumula length, the interaction of doses and varieties was found to be statistically significant (Table 1). While the highest plumule length was obtained from the control group, as the severity of salinity increased, the stem length value also decreased and got the lowest value at the highest salt dose of 200 ppm. When the effect of salt stress on cultivars was examined, all cultivars used in the experiment showed the best results in terms of plumule length in the control group. The Norman variety was the variety most affected by salinity, with the lowest value at 200 ppm. In the studies of the researchers on the seedling development of flax, it was stated that the radicle and plumule length of the seedlings were significantly affected by 150 and 200 mM salt concentrations (Zaghdoudi et al. 2015; Yaver and Pasa 2009; Muhammad and Hussain 2010; Kadkhodaie and Bagheri 2012). Moghaddam et al. (2018) reported that as the salinity intensity increases, plumule length decreases and plumule development slows down considerably at 12 dS/m salinity dose. Kadkhodaie and Bagheri (2012), in their study in which they examined the seedling growth parameters of flax seeds under salt stress, stated that flax seeds exposed to high concentrations showed a slowdown in plumule development.

In the study, seedling length was examined to determine the effects of salt doses on seedling development (Table 1). The effect of salt doses on the development of seedling length of flax seedlings was found to be statistically significant (P<0.05). When the effect of salt doses on cultivars was examined, it was observed that the highest seedling length was obtained from the control group, and as the salinity concentrations increased, significant decreases in seedling length occurred. At the highest salt concentration (200 mM), serious reductions in seedling length occurred and Midin variety was the variety with the lowest seedling growth. Kaya et al. (2012) studied the response of some linen lines to salt stress. In their study, they reported that varieties showed significant differences in terms of germination parameters at different salt doses and that seedling growth slowed down significantly at 20 dS/m, the highest salt concentration. El-Nakhlaway and El-Fawal (1989) reported that salt stress significantly reduced germination and seedling growth in flax plant development. The reason for the slowdown in germination is due to the osmotic stress that occurs with the effect of ion exchange at increasing salt levels (Almansouri et al. 2001; Kaya et al. 2006; Atak et al. 2006).

Table 1. *Radicle length, plumula length, seedling length values of flax seeds at different salt concentrations*

Radicle length (mm)				
Dose/ Variety	**Somme**	**Midin**	**Norman**	**Average**
Control	91.30^{a}	48.39^{cd}	52.67^{c}	64.12^{A}
50 mM	69.33^{b}	34.63^{ef}	40.95^{de}	48.30^{B}
100 mM	30.84^{fg}	22.15^{gh}	14.73^{hi}	22.57^{C}
200 mM	11.75^{i}	10.42^{i}	4.30^{j}	7.39^{D}
Average	50.80^{x}	28.89^{y}	27.09^{y}	
CV (%)	16.01			
LSD(0.05)	V: 4.47*	D:5.21*	V x D : 7.99*	
Standard Error	V: 1.650	D: 1.901	V x D: 3.301	
Plumula length (mm)				
Dose/Variety	**Somme**	**Midin**	**Norman**	**Average**
Control	103.09^{a}	74.93^{b}	105.02^{a}	94.35^{A}
50 mM	88.05^{ab}	45.31^{c}	79.51^{b}	70.96^{B}
100 mM	27.83^{cd}	29.12^{cd}	17.43^{de}	24.79^{C}
200 mM	10.75^{de}	11.10^{de}	3.90^{e}	7.28^{D}
Average	57.43	40.11	50.49	
CV (%)	24.62			
LSD(0.05)	V: N.S	D : 22.98*	V × D: 39.80**	
Standard Error	V: 3.269	D:4.878	V × D: 6.538	
Seedling length (mm)				
Dose /Variety	**Somme**	**Midin**	**Norman**	**Average**
Control	194.39^{a}	123.32^{c}	157.70^{b}	158.47^{A}
50 mM	157.39^{b}	79.94^{d}	120.46^{c}	119.26^{B}
100 mM	58.67^{de}	51.27^{ef}	32.16^{fg}	47.37^{C}
200 mM	22.50^{gh}	21.52^{gh}	7.81^{h}	14.67^{D}
Average	108.24^{x}	69.01^{y}	77.58^{y}	
CV (%)	19.56			
LSD(0,05)	V: 17.18*	D: 36.98*	V × D : 63.48*	
Standard Error	V: 4.224	D:4.878	V × D: 8.449	

* and **:significant at P ≤ 0.05 and P ≤ 0.01 respectively. N.S: No significant. a, b, c...; varieties × doses, A, B.C...; average of doses, x, y, z... ; average of varieties

The effects of salt doses on seedling fresh weight were given in Table 2 and it was determined that there was a statistical difference between salt doses in terms of seedling fresh weight (P<0.05). The effect of salt doses in the experiment on the seedling fresh weight of flax seedlings was investigated. It was determined that the highest fresh seedling weight was obtained from the control group and the seedling fresh weight decreased significantly as the intensity of the doses increased. Moghaddam et al. (2018) reported that seedling fresh weight in flax decreased significantly with increasing salinity doses, and seedling fresh weight was the highest in control and 50 mM. Similarly, Jamil et al. (2006) reported that the seedling fresh weight (shoot + root) of flax was affected by all salt doses (4.7-14.1 dS/m) and the shoot fresh weight decreased more than the root fresh weight. High salinity in the environment causes an increase in osmotic pressure. This situation causes imbalances in the water uptake of the seed and slows down both the germination of the seed and the development of the germinated seeds (Rajabi and Postini 2005). The increase in osmotic pressure (more negative osmotic pressure) caused by environmental salinity prevents the water uptake of the seed. This situation both disrupts the hydration balance of the seed and prevents seed germination by creating toxic effects of cations and anions in high amounts in the environment.

The effects of salt doses on germination rate are given in Table 2 and it was determined that there was a statistical difference between salt doses and varieties in terms of germination rate (P <0.01). Germination rate of all cultivars decreased with increasing salt doses. At the highest salt concentration (200 mM), the germination rate was severely reduced. Moghaddam et al. (2018) reported that different salt concentrations affect the germination parameters of flax, while increasing salt doses significantly affect germination. Many researchers have reported that the increase in salt levels has a negative effect on the germination of flax seeds (Jamil et al. 2006; Vicente et al. 2007; Mohammadizad et al. 2013). Abbasian and Moemeni (2013) explained the reason for the decrease in germination with the increase in salinity level, with the increase of toxic ions in the root region of the plant and the imbalance in the nutrient intake of the plant as a result of the increase in osmotic pressure. Knowing the degree of tolerance to salinity in the germination and early development stages of plants is an important parameter in terms of evaluating the growth and development of the plant. Many researchers have reported that salinity slows down root and shoot growth in plants and negatively affects germination (Vicente et al. 2004; Abdul Jaleel et al. 2007; Demir Kaya et al. 2012; Moghaddam et al. 2018; Büyükyıldız et al. 2023).

Table 2. Fresh seedling weight and germination rate values of flax seeds at different salt concentrations

Fresh seedling weight (mg)				
Dose /Variety	**Somme**	**Midin**	**Norman**	**Average**
Control	0.700^{a}	0.636^{b}	0.6333^{b}	0.656^{A}
50 mM	0.693^{a}	0.600^{b}	0.540^{c}	0.611^{B}
100 mM	0.410^{d}	0.303^{e}	0.263^{e}	0.325^{C}
200 mM	0.263^{e}	0.143^{f}	0.255^{g}	0.135^{D}
Average	0.516^{x}	0.359^{z}	0.420^{y}	
CV (%)	6.26			
LSD$_{(0.05)}$	V: 0.036*	D: 0.026*	V × D: 0.046*	
Standard Error	V: 0.007	D:0.009	V × D: 0.015	
Germination rate (%)				
Dose / Variety	**Somme**	**Midin**	**Norman**	**Average**
Control	92.88^{a}	92.55^{a}	91.22^{a}	92.21^{A}
50 mM	75.78^{ab}	52.33^{d}	67.77^{bc}	65.29^{B}
100 mM	60.00^{c}	28.11^{de}	40.00^{d}	43.70^{C}
200 mM	21.11^{ef}	17.77^{f}	4.49^{g}	12.96^{D}
Average	62.44^{x}	47.69^{y}	50.87^{y}	
CV (%)	14.80			
LSD$_{(0.05)}$	V: 9.02*	D: 8.78*	V × D :15.22**	
Standard Error	V: 2.131	D: 2.461	V × D: 4.263	

* and **:significant at $P \leq 0.05$ and $P \leq 0.01$ respectively. N.S: No significant. a, b, c...; varieties × doses, A, B...; average of doses, x, y, z... ; average of varieties

CONCLUSION

The purpose of evaluating plants in terms of salt resistance is to get an idea of which plants can and cannot be grown in salty soils. Salinity studies are carried out not only among plants but also between different varieties or genotypes of a plant. The effect of salt stress on cultivars was found to be significant in the study using three different flax seeds and four different salinity doses, including a control. It was observed that all properties examined in the study were adversely affected by high salt concentrations. It was determined that there were serious differences between the highest salt dose of 200 mM and the control group in which only pure water was used. In the study, the ‘Somme’ variety gave better (positive) results than other varieties in terms of germination parameters and seedling growth at high salinity doses. It was determined that the 200 mM salt dose significantly affected the germination of flax.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHOR CONTRIBUTION

All authors contributed equally.

ETHICAL APPROVAL

During the writing process of the study titled "**Investigation of Germination and Early Seedling Development of Some Flax (*Linum usitatissimum* L.) Seeds Under Salt Stress**", scientific rules, ethical and citation rules were followed; No falsification has been made on the collected data and this study has not been sent to any other academic media for evaluation. Ethics committee approval is not required.

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