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The Effect of Different Salinity Levels on Germination Development of Some Flax (*Linum usitatissimum* L.) Varieties

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A R T I C L E I N F O

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A B S T R A C T

Salinity, which is one of the abiotic stress factors, severely restricts plant production as a result of the negative effects of plants in different growth and development periods. Therefore, it is extremely important to determine the tolerance limits of plants to salinity in order to eliminate the limiting effect in terms of plant growth. Flax is an industrial plant that is used for multiple purposes and has commercial importance in the world. This research was carried out in controlled laboratory conditions in 2021 to determine the effects of salinity on the germination of flax seeds.
In the study, germination rate, root length, root fresh weight, shoot length and shoot fresh weight were evaluated. The result showed that significant differences between different NaCl solutions for all evaluated characters. Although the highest value was obtained in the control group in Mures variety, the highest value was obtained in 25 nM NaCl concentration in all other characters except for the germination rate in Dakota variety. The highest germination rate of 93.3% was obtained from the control application (0 mM NaCl) in both varieties. On the other hand, there was no germination in both varieties in 200 mM application.

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Introduction

The problem of global warming has caused irregularity in the amount of precipitation in recent years. Uneven distribution in precipitation, poor quality wastewater in agricultural production, especially in arid and semi-arid regions, unconscious practices such as drainage and improper and intensive use of river water cause soil salinity problems (Kiremit et al., 2017). Salinity in the soil is one of the important issues that can reduce the amount of production in irrigable areas as well as in arid regions. Soil salinity has also increased significantly in recent years due to various factors such as low precipitation, excessive irrigation, high evaporation and poor agricultural practices. In addition, excessive precipitation and high relative humidity in the summer months in recent years quickly remove soil water, increase evaporation and transpiration in plants, and increase the concentration of dissolved substances in the soil, causing salt accumulation. For these reasons, salinity in agricultural production areas is one of the most important problems affecting plant productivity negatively and limiting yield. Recent reports indicate that approximately 20% of total cultivated land and 33% of irrigated lands are currently affected by saline conditions, and by 2050 more than 50% of arable land will be saline (Shrivastava and Kumar, 2015).

Salt stress is an important abiotic stress factor that reduces the germination and vitality values of plants, even causes the plant to completely lose its vitality depending on the intensity and duration of the stress, and negatively affects many physiological events, especially photosynthesis and respiration. As a result, it causes losses in growth, development and yield (Bose et al., 2014). In almost all field crops, germination of seeds and primary growth of seedlings is the initial response to abiotic stress, and this response has an important role in the final product of the plant. Salinity stress in the plant primarily causes a decrease in germination and seedling formation (Almansouri et al., 2001).

Too much soluble salt in the soil solution reduces the water availability of plants. Increasing salinity makes the water and osmotic potential of plants more negative (Mugdal et al., 2010). This situation, which directly affects the rooting zone, affects the general water conditions of the plants. the plant with the loss of water in the soil, can absorb very little water from the soil and therefore the water potential is further reduced (Taiz et al, 2014). Varieties with resistance to salinity during germination ensure a plant is stable. Various reports indicate that seeds that are highly tolerant to salinity at the germination stage will have better growth at the seedling stage and will produce a stronger root system (Misra and Dwivedi, 2004).

Since removing salinity in soil and irrigation water is time consuming and costly, it is necessary to use and develop salinity-resistant varieties for the sustainability of agricultural production. In recent years, the demand for herbal products in both the food and textile industries and the increasing concerns about the sustainable use of resources has increased the interest in plants with relatively less input and versatile product potential in agricultural production.

Flax (*Linum usitatissimum* L.), a product that can meet the increasing demand for agricultural production, is a plant used in both fiber and oil production (Kurt and Bozkurt, 2006). Flax has been reported to be the source of three major classes of commercial products: fiber, oil, and bioproducts (Wang et al., 2012). In addition, it has been reported that the consumption of flax oil or flaxseed has significant beneficial effects in the prevention/treatment of some cancers, neurological and hormonal disorders, cardiovascular and inflammatory diseases (Kurt and Göre, 2021). This important industrial plant, known for its adaptation to poor soils and low input demand, requires investigation of the response mechanisms against high salinity. Various researchers have aimed to make flax more salt tolerant by many different methods, including traditional plant breeding and biotechnological approaches (Hashem et al., 2012, Kiremit et al., 2017). However, the successful cultivation of salt-tolerant flax varieties has not yet been declared. Flax has a relatively limited root system and needs sufficient moisture throughout the life period (Hocking et al., 1997). The water requirement of the flax, which uses the water in its body prudently, is relatively low compared to the plants in the same product group. It has been reported that germination rate, plant fresh weight, root and shoot length decrease in flax genotypes with increasing osmotic stress, and salinity stress causes a decrease in germination ratio (Almansouri et al., 2001; Asgharipour and Rafiei, 2010). In addition, it has been reported that increasing salinity levels cause a decrease in germination ratio, delay in emergence, and inhibition of seedling growth in different flax varieties (Sebei et al., 2007; Kaya et al., 2011). However, there is little information about the effects of salinity stress on germination, growth and germination parameters of flaxseeds.

When the effect of salt stress occurring during the germination phase is reduced, it is possible to grow an optimum number of healthy plants per unit area. As a result, the sustainability of production can be ensured by growing efficient and high quality products. Therefore, this research was carried out to determine the effects of salinity on the germination of flax seeds.

Materials and Methods

Plant Material

The experiment was carried out in April 2021 at Igdir University Research Laboratory Application and Research Center. Flax (*Linum usitassimum* L.) seeds, which are Mures and Dakota varieties that have adapted after sowing in Igdir, were used in this research.

NaCl Concentration and Treatments

The experiment was conducted with a randomized complete block design with 3 replications and placed 15 seeds in each petri dishes. Before sowing, the seeds were surface-sterilized with 3% Formaldehyde for 10 minutes and washed 5 times with re-distilled water. Then, sterilized filter papers in Petri dishes were moistened with 0 mM NaCl, 25 mM NaCl, 50 mM NaCl, 100 mM NaCl and 200 mM NaCl solutions prepared via distilled water. Petri dishes were covered with parafilm to prevent evaporation and minimize changes in solution concentration. They were kept in the growth chamber at 25ºC. Counting of germinated seeds was made every day until the last 10th day of the germination period. Germinated seeds were removed from Petri dishes and moisture was maintained by adding 5 ml of prepared salt solutions after every three-day period. After the experiment expire, 10 seedling samples were taken from each petri dish to measure root length and shoot height using a ruler (on a black background). Root fresh weight and shoot fresh weight were determined using precision scales. The germination rate (GR) was calculated according to the formula below.

$$GR = (\text{germinated seeds} / \text{total seeds}) \times 100.$$

Statistical Analysis

To evaluate the results obtained, a one-way analysis of variance (XLSTAT) which reveals whether there is a difference between the averages of the varieties was performed (Gomez and Gomez, 1984). Pearson correlation (r) was used to determine the relationship between the investigated parameters. Due to the number of varieties and were examined parameters, principal component analysis (Principal Component Analysis, PCA) (XLSTAT, 2021) was performed to eliminate the dependence structure of the relevant parameters and to reduce the dimension.

Results and Discussions

The experiment results showed that increasing salt doses caused significant reductions in flax seed germination ratio, root length, shoot length, root fresh weight and shoot fresh weight (Table 1). Germination period varies between 3 and 9 days, and the highest germination rate of 93.3% was obtained from the control application (0 mM NaCl) in both varieties. It was determined that the germination rate decreased in both varieties due to the increase in salt concentration, and this decrease was higher in Mures variety. On the other hand, there was no germination in both varieties in 200 mM application (Table 1).

Germination is generally the most sensitive stage to salt stress however, the level of salt tolerance can majorly change depending on varieties.

Table 1. Average data of germination parameters of two flax varieties at different salt concentrations

Variety	Treatments	Germination (%)	Root length (cm)	Shoot lenght (cm)	Root FW (g)	Shoot FW (g)
Dakota	0 Mm NaCl	93.3 ± 1.00^{A}	2.61 ± 0.77^{A}	0.98 ± 0.24^{B}	0.015 ± 0.005^{AB}	0.030 ± 0.01^{A}
	25 mM NaCl	73.3 ± 1.00^{B}	3.28 ± 0.60^{A}	1.43 ± 0.27^{A}	0.02 ± 0.01^{A}	0.035 ± 0.015^{A}
	50 mM NaCl	56.6 ± 0.50^{C}	1.54 ± 0.22^{B}	0.65 ± 0.03^{C}	0.01 ± 0.00^{B}	0.020 ± 0.00^{A}
	100 mM NaCl	36.6 ± 0.41^{D}	0.81 ± 0.40^{BC}	0.62 ± 0.032^{C}	0.00 ± 0.00^{C}	0.00 ± 0.00^{B}
	200 mM NaCl	0.00 ± 0.00^{E}	0.00 ± 0.00^{C}	0.00 ± 0.00^{D}	0.00 ± 0.00^{C}	0.00 ± 0.00^{B}
Statistics		df=14; F=172.95; P=0.00	df =14; F=21.202; P =0.00	df =14; F=31.12; P =0.00	df =14; F=9.600; P =0.02	df =14; F=12.462; P =0.01
Variety	Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Root FW (g)	Shoot FW (g)
Mures	0 Mm NaCl	93.3 ± 0.82^{A}	2.98 ± 0.11^{A}	1.71 ± 0.53^{A}	0.02 ± 0.008^{A}	0.045 ± 0.012^{A}
	25 mM NaCl	76.6 ± 0.41^{B}	2.38 ± 0.49^{B}	1.06 ± 0.097^{B}	0.02 ± 0.008^{A}	0.040 ± 0.008^{A}
	50 mM NaCl	33.3 ± 0.81^{C}	0.78 ± 0.073^{C}	0.26 ± 0.016^{C}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}
	100 mM NaCl	16.6 ± 0.41^{D}	0.47 ± 0.183^{D}	0.20 ± 0.006^{C}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}
	200 mM NaCl	0.00 ± 0.00^{E}	0.00 ± 0.00^{E}	0.00 ± 0.00^{D}	0.00 ± 0.00^{B}	0.00 ± 0.00^{B}
Statistics		df=14; F=212.55; P=0.00	df =14; F=303.988; P =0.00	df =14; F=17.51; P =0.00	df =14; F=9.00; P =0.02	df =14; F=25.154; P =0.00

The germination rate is one of the important features of the base of seed which can be measured as a limiting factor in the plant. The seeds which grow rapidly will have less exposure to pest and disease attacks (Sebei et al., 2007). So, the seedling is able to have better and proper use of the environmental factors and damage at the initial stage of saline agricultural lands will be minimized. This research revealed that the negative significant effect of salinity stress on flaxseed germination percentage up to 50 mM NaCl is within acceptable limits and that flaxseed can show a certain tolerance to salinity stress up to this concentration.

The longest root length (2.98 cm) in Mures variety was obtained in the control application, and it was determined that the root length decreased as the salt concentration increased. In Dakota variety, it was determined that the root length increased (3.28 cm) up to 25 mM NaCl salt concentration and the root length decreased at higher salt concentrations (Table 1). The root length obtained during the germination period is an important indicator of the plant's attachment to the soil and thus its survival in the face of stress conditions. Zaghdoudi et al. (2015) reported that root length decreased by 86% when flax varieties were treated with 250 mM NaCl. Similarly, it has been revealed by various researchers that the root length decreases depending on the increase in the salinity level (Kara et al., 2011; Benlioğlu and Özkan, 2015). Under the salinity stress, the root length feature had less decrease with salinity levels increase after 25 mM NaCl in comparison with the untreated seeds for Dakota varieties. However, the root length of Mures variety showed a regular decrease as the salt level increased. In the previous research of linseed varieties, reactions against salinity showed that the root length is a proper criterion to determining the resistance varieties against salinity stress. (Kiremit et al., 2017).

As a result of the research, the longest shoot length (1.71 cm) in Mures variety was obtained in the control application, and it was determined that the shoot length decreased as the salt concentration increased. In the Dakota variety, it was determined that the shoot length increased (1.43 cm) up to 25 mM NaCl salt concentration, and the stoot length decreased at higher salt concentrations. When the two flax varieties are compared in terms of shoot length; It was determined that Mures variety was more affected by 25, 50 and 100 mM NaCl salt concentrations (Table 1). These findings were also found to be close with Robin et al. (2016) and Rahneshan et al. (2018). Because root and shoot length are regarded major indicators of plant response to environmental stress, they may be used to assess plant stress tolerance capacity. The first impacts of salinity cause a decrease in early plant development owing to a lack of water. Simultaneously, salt stress causes a nutritional imbalance in plants, resulting in a decrease in plant nutrient absorption and development (Singh et al., 2020). The results of this study show that salt stress reduces root and shoot length in all linseed genotypes. This reduction in growth may be due to the negative effects of the high osmotic potential of the soil solution, which reduces the absorption of water and nutrients and ultimately reduces root and shoot growth.

This study also showed that salinity had a negative effect on root and shoot fresh weight, as in other evaluated characters. In Dakota flax variety, although both characters were positively affected up to 25 mM NaCl salt concentration, they were negatively affected by higher salt concentrations. In Mures variety, both characters were adversely affected by all other applications except the control application (Table 1). As a matter of fact, Kaya et al. (2005) reported that there may be an increase in root fresh weight of some plants at low NaCl salt levels. Root and shoot FW are one of the most important characteristics of salt stress due to its direct contact with the soil. The reasons for the decrease in the growth and functions of plants under salt stress, (1) change in the transfer of photosynthesis products to the roots, (2) decrease in shoot growth, (3) partial or complete occlusion of stomata, and (4) effects of salt on shoot photosynthesis and ion balance. effects. (Heidari, 2009). Furthermore, in salinity stress environments, a reduction of water absorption via the seed leads in a decrease in hormones and enzymes, causing a difficulty with root, shoot, and seedling growth (Kadkhodaie and Bagheri, 2011).

Correlation indicators increases the possibility of indirect selection for different parameters. This shows information to the researcher about importance of any parameters and its relations.

It was determined that the relations between all parameters of both varieties evaluated in the study were statistically significant and positive. In addition, it was determined that the closest correlation relationship between the evaluated characters of both varieties was between root FW and shoot FW (0,993 and 0,979). On the other hand, interestingly, the correlation values between root length and root weight (0.934 and 0.840) and between shoot length and shoot weight (0.870 and 0.954) are lower than the correlation values between these two characters two characters in Dakota and Mures flax varieties (Table 2). These positive important and closely related correlations showed that flax varieties contributed to growth and development in relation to each other under salt stress conditions.

As a result of the research, it was determined that 69.07% of the total variance was formed by the first principal components (PC1) and 29.87% by the second principal components (PC2). When PCA results are evaluated; Among the evaluated features, it was determined that the stem fresh weight and germination rate differed from other characters (root length, root fresh weight and shoot length). In addition, PCA revealed that the effects of increasing salt concentrations on stem fresh weight and germination rate were less than the effects on root length, root fresh weight and shoot length. In other words, all salt concentrations above the 25 mM NaCl salt concentration had a more limiting effect on all evaluated characters. It was determined that this effect appeared more clearly on stem length, root length and root fresh weight (Figure 1).

Table 2. Correlation coefficients of germination parameters of two flax varieties at different salt concentrations

Dokato Variety	Germination rate	Root length	Shoot lenght	Root FW	Shoot FW
Germination rate	1				
Root length	0.892672**	1			
Shoot lenght	0.848256**	0.951146**	1		
Root FW	0.801099**	0.934252**	0.879157**	1	
Shoot FW	0.843938**	0.941378**	0.869514**	0.993299**	1
Mures Variety	**Germination rate**	**Root length**	**Shoot lenght**	**Root FW**	**Shoot FW**
Germination rate	1				
Root length	0.958144**	1			
Shoot lenght	0.91585**	0.903986**	1		
Root FW	0.84083**	0.839943**	0.911953**	1	
Shoot FW	0.913833**	0.915822**	0.953977**	0.978761**	1

**: significant

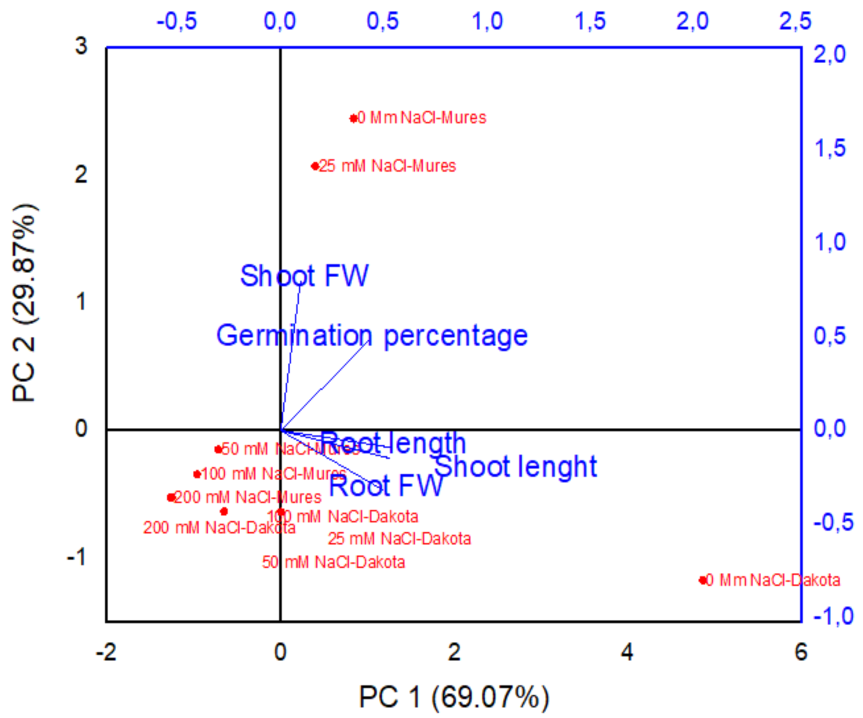

Figure 1. Principles component analysis (PCA) of germination parameters of two flax varieties at different salt concentrations

Conclusion

The results of this study suggest that, the effects of environments with different irrigation water salinity levels on flax germination and some seedling growth parameters were investigated. The highest germination rate values were obtained from 73.3% Dakota variety and 76.6% Mures variety from 25 mM NaCl treatments except control groups. It was determined that the salt concentration created a stress condition for all parameters examined after 50 mM NaCl level for Dakota variety and 25 mM NaCl level for Mures variety. These controlled-environment results should be supported by longer and more thorough field research. Considering the areas where salinity stress has expanded due to global warming and changes in precipitation patterns, it is understood that the flax plant has a limited chance to grow in areas with such stress conditions. In addition, it has been determined that the varieties to be grown should be taken into account in order to better evaluate this potential.

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Conflicts of Interest

The authors declare no conflict of interest.

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