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Araştırma Makalesi / Research Article

Determination of Tolerances of Some Flax Varieties to Different Doses of Salt Concentrations in Early Development Period

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Abstract

It is widely accepted that soil salinity increases over time and is triggered by the effect of climate change. The aim of this research is to examine the germination, growth response and tolerance of some flax (*Linum usitatissimum* L.) varieties of different salinity levels. This study was carried out with 11 flax genotypes in 2022 in the germination laboratory of Muş Alparslan University, Faculty of Applied Sciences, Plant Production and Technologies. The research was established according to the Randomized Plots Trial Design with 4 replications, and 0, 75, 150 and 225 mM NaCl salt solution levels were applied. In the analysis of variance, genotype, salt levels and genotype*salt level interaction were found to be statistically significant at the P <0.01 level in terms of all the traits examined. In all cultivars used in the study, 75 mM NaCl concentration showed a positive stimulating effect on germination. However, although 150 mM NaCl concentration had a germination-promoting effect, it negatively affected normal seedling formation. As a result, when the anatomical, physiological and biochemical findings of flax varieties are evaluated as a whole in the early development period; It was determined that the most sensitive cultivars to salinity were G2, G10, and G11 varieties, moderately sensitive varieties were G1, G4, G5 and G8, in terms of all doses, the most stable variety was G3, in addition G6, G7 and G9 of varieties showed good results in terms of all traits. However, it was concluded that these varieties should be cultivated under field conditions in order to obtain more decisive results.

Keywords: Flax, NaCl, germination seed, correlation, tolererence, proline, MDA

1. Introductıon

Flax (*Linum usitatissimum* L.) is a multi-purpose plant due to its many benefits in both human and animal nutrition. Flax is the oldest agricultural product, which has more than 300 species and has been cultivated since ancient times. 3.4 million tons of flax seeds are produced in the world. The countries that produce the most flax seeds in the world are Kazakhstan, the Russian Federation, Canada, China and the USA. The largest flaxseed producer in the world is Kazakhstan with 1.1 million tons, which constitutes approximately 31% of the total production in the world (FAOSTAT, 2020).

Salinity is one of the limiting factors of plant productivity and quality as well as affecting various physiological processes. However, it is at the forefront of environmental struggles that limit agricultural production worldwide. (Shrivastava and Kumar, 2015; Yamaguchi and Blumwald, 2005). It is widely accepted that soil salinity increases over time and is triggered by the effect of climate change. Although the latest statistics on the global extent of soil salinity are not available; Various scientists have reported that 10% of the total arable land is affected by salinity and alkalinity, and FAO has reported that 932 million hectares of land worldwide are affected by salt. (Shahid et al., 2018). Future projections until 2050 indicate that nearly half of the arable land may be adversely affected by salt stress (Bilmez-Özçınar, 2021).

Salt stress in plants can be explained by the fact that the salt in the soil makes it difficult for the roots to take water, and the salt causes a toxic effect on the plant. (Misra and Dwivedi, 2004). Na+ ion taken into the plant as a result of salt stress disrupts the entry of ions such as K+ and Ca+2, which are essential for the natural development of plants, and affects the ion balance in the cell, causing the ionic balance of the cells to deteriorate (Yokoi et al., 2002). In addition, excessive salinity negatively affects the stability of the membrane system by secreting sterols in the cell membrane (Reddy and Iyengar, 1999) and in parallel with the rate of ROS formation, it causes the structure of the double-chain lipids in the membrane to deteriorate (Parida and Das, 2005). Salt stress has negative effects on PS I, PS II and stomatal conductivity of plants and causes a decrease in CO_2 assimilation during photosynthesis, causing disruptions in organic matter synthesis and developmental disorders. (Asada, 1999; Apel and Hirt, 2004). Salinity either inhibits germination completely or slows growth at higher or lower levels (Iqbal et al., 2006). Salinity has effects on seed germination through osmotic effects and specific ion toxicity (Huang ve Redman, 1995; Song et al., 2009). Many researchers reported that salinity delays and reduces germination, root and shoot growth. (Vicente et al., 2004; Ashraf, 2004; Jaleel et al., 2007). Many researchers, Sorghum (*Sorghum bicolor* L.) (Rajabi Dehnavi et al., 2020), Lentil (*Lens culinaris* Medik.) (Phot et al., 2019), Foxtail millet (*Setaria italica* L.) (Pan et al., 2020), Faba bean (*Vicia faba* L.) (Yang et al., 2020) examined the effect of salinity on plant germination.

Poor germination and seedling growth in arid and semi-arid conditions are a major problem and are considered the main factors to be considered in the later development and yield of the plant (Li et al., 2011).

The flax plant is known to be more sensitive to saline soils than most other cultivated plants. Salt stress tolerance of plants is affected by many factors, but it is also important to evaluate salt tolerance on the basis of variety (Assaha et al., 2017; Chuamnakthong et al., 2019). For this reason, determination of salinity-tolerant genotypes and selection of varieties in production areas are very important in order to minimize the losses in production potential in arid and semi-arid areas where salinity is high. Bu çalışma, 11 keten genotipinin ilk gelişim dönemindeki tuzluluk toleransını belirlemek amacıyla yapılmıştır.

2. Materials nnd Methods

2.1. Materials

This study was carried out in the central laboratory of Muş Alparslan University in 2022 to reveal the response of some flax genotypes to salt (NaCl) stress during germination and early growth periods. The study was applied to the seeds of 11 flax genotypes with 4 different concentrations of NaCl salt (0, 75, 150 and 225 mM) and was set up in 4 replications according to the Randomized Plots Trial Design. Information on the varieties is given in Table 1.

Table 1. Some information about the varieties

codes of genotypes	genotype name	country of orjin	growing type	1000 seeds weight (g)	oil rate (%)
G1	Sarı 85*	Türkiye	Spring	5.40	37.7
G2	Dillman*	U.S.A	Winter	5.20	32.3
G3	Olin*	Serbia	Spring	6.80	36.0
G4	Larnaka*	Pakistan	Winter	5.40	37.3
G5	Tash*	Afghanistan	Spring	4.20	34.4
G6	Clli-1400*	Türkiye	Winter	5.90	38.2
G7	Milas*	Türkiye	Winter	6.20	36.1
G8	Lala Musa*	Pakistan	Spring	4.90	36.3
G9	Antares*	France	Spring	5.9	35.7
G10	Bombay*	India	Spring	4.6	35.5
G11	Nored*	U.S.A	Spring	3.8	34.0

*The seeds used in the experiment were supplied by the United States Department of Agriculture (USDA).

2.2. Methods

In this study; The used petri dishes (9 cm in diameter) and were left in the autoclave (120 ^{o}C and 20 minutes) for salt sterilization. In the sterilization process of the seeds, the seeds were kept in 70% ethanol for 10 seconds and then kept for 1 minute with sodium hypochlorite. It was then washed with distilled water 4 times. 25 seeds of each variety were sown in petri dishes with double layer blotting paper, evenly spaced, with the help of tweezers. 7 ml of distilled water for the control group and 7 ml of NaCl (0, 75, 150 and 225 nM) solution adjusted to other petri dishes were added to each petri dish. Petri dishes were covered with parafilm to prevent evaporation. In accordance with ISTA rules, it was kept at 25 ^{0}C and 65% humidity in a dark environment for 24 hours. During the experiment, seeds germinated every day (1 in 24 hours) were counted and seeds with a root length of 2 mm were considered germinated. The experiment was terminated on the 9th day and the root and shoot lengths of 10 randomly germinated seeds from each petri dish were measured with a digital caliper and averaged. Roots and shoots were kept in an oven at 70 ^{o}C for 72 hours and root and shoot weights were measured on a balance with a sensitivity of 0.0001 g. According to ISTA rules, seedlings with well-developed, complete, proportionate and healthy seedlings were considered normal seedlings, and seedlings with poor growth, physiological disorders, or deformed or disproportionate basic structures were considered abnormal seedlings. (ISTA, 2020). Among different varieties of the same species, it is known that the resistant variety is classified as high in hydrogen peroxide content, and the variety with low hydrogen peroxide content is classified as susceptible. Saglam et al. (2014) in a study on different varieties of maize, it was stated that the hydrogen peroxide content of the resistant variety was significantly higher than the susceptible variety. In the current study, those with high hydrogen peroxide content, low MDA content and the least increase in proline content under stress were accepted as

resistant. Among the control groups, those with low hydrogen peroxide content, relatively high MDA content under stress, and the highest increase in proline content under stress were considered as susceptible varieties.

2.2.1. GP (germination percentage)

The percentage of germination was calculated according to the formula given below (Fang et al., 2006).

G% = *n/N x* 100

N: the sum of germinated seeds and N: the total number of seeds in the test. Germination percentage: number of germinated seeds/total number of seeds in the test.

2.2.2. Rs (germination rate)

Germination rate was calculated according to the formula given below (Datta and Dayal; 1991)

$$R_s = \sum_{i=1}^{n} S_i \,/\, D_i$$

R_s: germination rate, Si: the number of germinated seeds on the counting day, Di: where n is the number of days until the counting day and n is the number of days counted.

2.2.3. MGT (mean germination time)

Mean germination time was calculated according to the formula given below (Ellis and Roberts, 1981). f: The germinated seed on the counting day, x: The number of counting days.

$$MGT=\Sigma(fx)/\Sigma f$$

2.2.4. SVI (seed viability index)

Seed viability index was calculated according to the formula given below. Moghadam et al. (2018). G: % germination percentage and SL average seedling length.

$$SVI = G\% \; x \; SL(mm)/100$$

2.2.5. Salinity tolerance index

The following equation was used to calculate the salt tolerance index (TTI) of flax seeds according to their salinity levels.

$$STI\ (\%) = \frac{T_x \text{ (root+shoot dry weight)}}{To(kontrol)(root+shootdryweight)} \times 100$$

T_x: root+shoot dry weight at NaCl dosing

T_0: root+shoot dry weights at control dosing

Then, germination percentage, mean germination time, germination rate, root and shoot length, root and shoot dry and fresh weights, seed viability index, salinity tolerance index were calculated.

2.2.6. Determination of proline content

0.1 g of fresh samples was taken and 1.8 mL of 3% sulfosalicylic acid was added to it and homogenized with tissue shredder. The homogenate was centrifuged at 5,000 rpm for 5 min at room temperature. 1 ml of supernatant was taken and 1 ml of acetic acid and 1 ml of ninhydrin were placed on it. The samples in the tubes were kept in a water bath at 100 ºC for 1 hour and the reaction was terminated on ice. 3 mL of toluene was added to the samples and vortexed. The supernatant was read in the spectrophotometer at 520 nm (Bates et al., 1973). Results are given as µg per gram fresh weight (TA).

2.2.7.Determination of lipid peroxidation

Lipid peroxidation level was determined according to the method of Heath and Packer (1968). 0.1 g of the samples were taken and 0.1% trichloroacetic acid (TCA) was added on it and homogenized with a tissue shredder. The homogenate was centrifuged at 15000 g for 5 minutes. After adding 4 mL of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA to 1 mL of the supernatant, the absorbance of the supernatant was recorded at 532 nm and 600 nm.

2.2.8. Determination of hydrogen peroxide (H_2O_2) content

H_2O_2 content was determined according to the method of Velikova et al. (2000). 0.1 g of the samples were taken and homogenized in 1.8 mL of 0.1% TCA with tissue shredder. The homogenate was centrifuged at 15,000 g at +4 °C for 15 minutes. After 1000 µL of the supernatant

was taken and 1000 µL of 10 mM potassium phosphate buffer and 1500 µL of 1 M KI were added, the yellow color formed was read from the registered standard graphic in the spectrophotometer at 390 nm.

2.3. Statistical analysis

Combined analysis of variance of the data obtained from the study was performed using the JMP Pro 13 package program and the factors found to be significant ($P < 0.05$, $P<0.01$) were evaluated and grouped according to the LSD test.

3. Result and Discussion

According to the combined variance analysis results; genotype, location and genotype×location The variance analysis values of the traits examined in the study are given in Table 2, and it was found to be statistically significant at the 1% level in terms of genotype, levels and genotype*levels interaction for all the traits examined (Table 2). In addition, germination percentage, number of normal and abnormal seedlings are in Table 3, average germination time and germination rate are in Table 4, seed viability index and salt tolerance index are in Table 5, Root and shoot lengths are in Table 6, root and shoot age weights are given in Table 7, root and shoot dry weights are given in Table 8, and the correlation values of the bilateral relations between the examined traits are given in Table 9. In terms of all traits studied, 225 mM salt concentration caused the highest inhibitory effect in all varieties.

Table 2. Variance analysis table for the examined traits

Germination Tratits/DF	Variation Source							
	Model	Genotip	Levels	Gen*Lev.	Error 1	Error 2	C. Total	CV(%)
	76	10	3	30	33	99	175	
GP	39269.159	3496.48**	63.8845**	120.384**	15.1875	1481.563	40750.72	8.59
Rs	71.47735	5.01257**	3.4214**	0.34285**	0.0243	3.081173	74.55853	9.83
MGT	9.949846	0.30447**	1.76337**	0.03744**	0.01491	1.454024	11.40387	2.67
SVI	70606.22	3098.33**	9611.35**	349.209**	9.47272	1175.579	71781.8	9.45
STI	126270.76	1199.62**	22246.9**	1502.04**	74.9221	2252.21	128522.97	5.08
RL	19066.88	516.207**	2261.83**	230.946**	5.78589	501.996	19568.88	6.94
SL	130048.6	734.606**	39448.3**	135.643**	8.73361	612.85	130661.4	4.69
RFW	0.104951	0.00309**	0.01933**	0.0005**	3.47E-05	0.002499	0.10745	9.50
SFW	3.918764	0.07972**	0.81222**	0.02248**	0.00032	0.019247	3.93801	4.93
RDW	0.001021	0.0000384**	0.00014**	0.0000071**	1.32E-07	1.49E-05	0.001036	7.71
SDW	0.014906	0.0005**	0.0024**	0.0000875**	1.68E-06	0.000392	0.015298	8.18
NP	2220.284	81.9659**	342.218**	11.4553**	0.91856	49.4375	2269.722	9.37
ANP	1844.921	64.3295**	278.536**	11.9402**	0.23674	22.4375	1867.358	12.20

**, p<0.01, *0.01<P<0.05, CV: coefficient of variation; DF: degrees of freedom, GP: Germination percentage, MGT: Mean germination time, Rs: Germination rate, SVI: Seed variability index, RL: root length, SL: shoot length, STI: salinity tolerance index, RFW: root fresh weight, SFW: shoot fresh weight, RDW: root dry weight, SDW: shoot dry weight, NP: normal plants, ANP: abnormal plants.

3.1. Germination percentage, normal and abnormal seedling percentage

In terms of germination percentage of varieties, normal and abnormal seedling numbers; Genotype, levels and genotype*levels interaction were found to be statistically significant at the 1% level (Table 2). Germination percentage decreased as NaCl concentration increased in all cultivars used in the study. The decrease in germination with increasing salinity level is thought to be probably due to osmotic potential, high toxic ions and seed nutrient imbalance (Ashkan and Jalal, 2013). In addition to the toxic effects of some ions, the higher salt concentration also reduces the water potential in the medium, which inhibits water absorption by the germination of seeds, and is thus hypothesized to be effective in reducing germination (Maas and Nieman, 1978). Many researchers have explained that this reduction in germination of seeds is a result of plants being exposed to salt stress

(Vicente et al., 2004; Ashkan and Jalal, 2013; Moghaddam et al., 2018; Rajabi et al., 2020, Fot, et al., 2019; Yang et al., 2020). 75 mM NaCl concentration showed germination promoting effect in all varieties used in the study. 150 mM NaCl dose from the application dose showed germination promoting effect, but increased the abnormal seedling percentage and gave negative results. Salt concentrations of 225 mM NaCl caused an increase in the percentage of abnormal seedlings in genotypes. Percentage of abnormal seedlings provides important data to see the effect of salt stress on the early growth stages of the plant. (Khayamim et al., 2014). Poor germination and abnormal seedling growth are recognized as a major problem to be considered in the later development and yield of the plant (Li et al., 2011). When germination percentage, normal seedling and abnormal seedling characteristics are evaluated together; The most sensitive variety to salinity is G11, followed by G2 and G10 varieties, respectively. It was determined that the varieties that are moderately sensitive to salinity are G1, G4, G5, G6, G7, G8 and G9, respectively, and the most stable variety to salinity is G3. Although the tolerance of plants to salt stress is affected by many factors, it is also important to evaluate salt tolerance on the basis of variety (Assaha et al., 2017; Chuamnakthong et al., 2019).

Table 3. Germination percentage, averages of normal and abnormal seedling number traits and resulting groups

Genotypes	GP(% NaCl Levels					NP(%) NaCl Levels					ANP(%) NaCl Levels				
	0	75	150	225	Mean	0	75	150	225	Mean	0	75	150	225	Mean
G1	59c-e	64c	57de	57de	59.2B	13.3bc	14.3a	9.0g-ı	9.3gh	11.4A	0.8rs	2.0op	5.3fg	5.8f	3.4DE
G2	34op	37l-p	39k-o	51fg	40.3EF	8.3ı-k	7.8j-l	5.3p-r	4.3st	6.4F	0.3s	1.5pq	4.5hı	8.5c	3.7CD
G3	43ı-k	54ef	50f-h	42ı-l	47.3D	8.5h-j	10.5ef	8.8hı	4.0t	7.9CD	3.8jk	3.0lm	3.8jk	6.5e	4.3B
G4	33pq	35n-p	41j-m	39k-o	37.0G	8.3ı-k	6.8mn	7.5km	4.3st	6.7EF	0.5s	2.8mn	3.8jk	5.5f	3.1E
G5	54ef	48gh	51fg	40j-n	48.4D	12.8cd	10.3ef	9.8fg	1.0w	8.4C	1.5pq	2.3no	3.0lm	9.3b	4.0BC
G6	38k-p	38k-p	40j-n	47g-ı	40.8E	8.5h-j	8.8hı	7.6lm	4.8q-t	7.3DE	2.0op	2.3no	4.0ıj	7.3d	3.9BC
G7	59c-e	57de	61cd	45h-j	55.5C	12.8cd	13.0bc	12.0d	2.5u	10.1B	1.5pq	2.0op	3.3k-m	9.0bc	3.9BC
G8	37l-p	43ı-k	38k-p	33pq	37.8FG	7.5k-m	9.8fg	7.5km	2.3uv	6.8EF	1.5pq	3.5j-l	2.0op	5.5f	3.1E
G9	75b	74b	70b	87a	76.5A	13.8ab	11.0e	8.8hı	4.5r-t	9.5B	4.8gh	7.0de	8.8bc	17.5a	9.5A
G10	26rs	28qr	36m-p	28qr	29.5H	5.8op	5.5o-q	6.3no	2.2x	4.4G	0.5s	1.5pq	5.3fg	1.3qr	2.1F
G11	20t	22st	26.5rs	25r-t	23.4I	4.3st	5.0p-s	5.5o-q	1.5vw	4.1G	0.5s	0.5s	1.8o-q	4.8gh	1.9F
Mean	43.5 B	45.5 A	46.3 A	44.9A B		9.4 A	9.3 A	8.0 B	3.5 C		1.6 D	2.6 C	4.1 B	7.3 A	

3.2.Mean germination time and germination rate

In terms of mean germination time and germination rates of varieties; Genotype, levels and genotype*levels interaction were found to be statistically significant at the 1% level (Table 2). It was determined that there was a gradual increase in the mean germination time as the NaCl concentration increased in all varieties used in the study. It is observed that the average germination time increases at the most 225 mM NaCl concentration. In parallel with the increase in NaCl levels, the germination rate decreased. The decrease in germination rate was the highest in G10 and G11 varieties, and the least in G9 varieties. G1 variety germinated in the longest time, while G6 germinated in the shortest time. Moghammad et al. (2018) reported that the germination rate decreased as salt stress increased.

Table 4. Mean germination time and germination rate traits and the resulting groups

Genotypes	MGT NaCl Levels					Rs NaCl Levels				
	0	75	150	225	Mean	0	75	150	225	Mean
G1	4.61f-ı	4.57g-l	4.83cd	5.37a	4.84A	2.13f-h	2.40de	1.72j-l	1.12s-u	1.84D
G2	4.48h-m	4.45ı-o	4.54g-m	4.64e-h	4.53CD	1.39o-r	1.59k-o	1.50l-q	1.81ı-k	1.57E
G3	4.40l-r	4.44j-p	4.48h-m	4.65e-g	4.50DE	1.97hı	2.29ef	2.04g-ı	1.51l-q	1.95D
G4	4.30n-s	4.47ı-n	4.53g-m	4.80c-e	4.52CD	1.62k-o	1.43n-r	1.59k-o	1.26q-s	1.48EF
G5	4.17s	4.41l-r	4.49g-m	4.75d-f	4.45DE	3.00b	2.25e-g	2.09f-h	1.31p-s	2.16C
G6	4.14s	4.27p-s	4.29o-s	4.61f-ı	4.33F	2.14f-h	1.95h-j	1.99h-ı	1.69k-m	1.94D
G7	4.25rs	4.29o-s	4.49g-m	4.60f-j	4.41EF	3.02b	2.82bc	2.46de	1.65k-n	2.49B
G8	4.51g-m	4.56g-l	4.55g-m	4.94bc	4.64B	1.51l-p	1.61k-o	1.46m-q	0.98t-v	1.39F
G9	4.38m-r	4.56g-l	4.59f-k	5.00b	4.63B	3.43a	2.84b	2.59cd	2.39de	2.81A
G10	4.44j-p	4.44ı-o	4.54g-m	5.01b	4.61BC	1.10s-u	1.19r-t	1.38o-r	0.78v	1.11G
G11	4.26q-s	4.43k-q	4.58f-k	4.64e-h	4.48DE	0.99t-v	0.96t-v	1.09s-u	0.89uv	0.98H
Mean	4.36 D	4.44 C	4.54 B	4.82 A		2.03 A	1.94 B	1.81 C	1.40 D	

3.3. Seed viability index and salinity tolerance index

In terms of seed viability index and salinity tolerance index; Genotype, levels and genotype*levels interaction were found to be statistically significant at the 1% level (Table 2). Seed viability index decreased in parallel with the increase in NaCl levels. The maximum decrease in seed viability index was observed in G2, G10 and G11 varieties, while the least decrease was observed in G5, G7 and G9 varieties. The effect of salinity in seeds either prevents water uptake by osmotic pressure or reduces the seed viability index by making a toxic effect on embryo viability (Houle et al., 2001). The most stable varieties in the salinitiy tolerance index were found to be G7 and G9.

Table 5. Means and groups of seed viability index and salinity tolerance index traits

Genotypes	SVI NaCl Levels					STI NaCl Levels				
	0	75	150	225	Mean	0	75	150	225	Mean
G1	58.65de	47.01gh	34.55n	18.20q-s	39.60D	100.0k	113.3d-g	90.4l	49.6qr	88.3EF
G2	36.17l-n	35.68mn	26.28o-p	21.88p-r	30.00F	100.0k	102.4ı-k	69.4o	60.3p	83.0F
G3	44.10hı	43.92hı	40.58ı-l	25.51op	38.53DE	100.0k	133.8b	114.5df	39.2s	96.9BC
G4	50.67fg	39.44ı-m	38.82k-n	17.85rs	36.70E	100.0k	85.4lm	107.3g-j	69.7o	90.6DE
G5	82.75b	43.77h-j	38.80k-n	14.99st	45.08C	100.0k	108.4f-j	110.5e-h	17.4t	84.1F
G6	51.85f	42.72h-k	38.97j-n	23.85op	39.35D	100.0k	107.4g-j	117.8d	75.4no	100.2A-C
G7	93.81a	60.18cd	54.15ef	22.74o-q	57.72A	100.0k	117.6d	126.5c	35.1s	94.8CD
G8	46.21gh	38.35k-n	27.36o	14.52st	31.61F	100.0k	142.3a	115.3de	53.4q	102.8AB
G9	82.56b	64.14c	35.6mn	34.28n	54.16B	100.0k	103.2ı-k	79.4mn	46.0r	82.2F
G10	25.16op	26.77o	10.71t	3.58u	16.56G	100.0k	100.2k	102.3jk	113.0d-g	103.9A
G11	15.97s	14.82st	10.60t	5.42u	11.70H	100.0k	104.1h-k	107.8h-j	109.0e-ı	105.2A
Mean	53.44 A	41.53 B	32.41 C	18.44 D		100.0 C	110.7 A	103.7 B	60.7 D	

3.4. Length, dry and fresh weight of roots and shoots

In terms of root and shoot lengths, dry and fresh weights; Genotype, levels and genotype*levels interaction were found to be statistically significant at the 1% level (Table 2). Root and shoot lengths are known as the most important parameters in explaining salt stress. Root and shoot length provide important clues in measuring the

plant's response to salt stress, as the roots absorb water from the soil and transmit it to other parts of the plant (Jamil and Rha, 2007; Moghammad et al., 2018). In terms of the varieties used in the study, NaCl promoted root elongation up to a certain level. Depending on the salt stress, the root length increased up to 150 mM NaCl level and decreased at the next level. On the basis of all varieties used in the study, root length was observed at the maximum 150 mM NaCl level and at least 225 mM NaCl level. Depending on the salt stress, root length was determined the most in G4 variety and at least in G2 and G3 variety. Although root length changes depending on the increase in salt stress, it is known that it is a feature that can change depending on the genetic structure. The shoots were shortened due to the increase in NaCl levels applied to the varieties. The shoots were shortened by the application of up to 225 mM NaCl. According to the averages of the varieties, the longest shoots were obtained in G7 and the shortest shoots were obtained in G1 varieties. Mohammad et al., (2018); reported a decrease in root and shoot lengths as NaCl levels increased. When evaluated in terms of varieties; Root and shoot weights shorten as NaCl levels increase, while dry and fresh weights decrease. The highest root and shoot number and fresh and dry weights were determined in G7 variety. In general, the growth of the seedlings was slowed down due to the increase in the salt level. It has been reported that these negativities caused by seedling development are caused by negative osmotic pressure disrupting the hydration of the seed and preventing the germination of seeds due to physiochemical toxicity (Moghammad et al., 2018; Rajabi et al., 2020). It has been reported that insufficient development of roots and shoots may occur due to salt toxicity as well as unbalanced nutrient intake and the ability of seedlings to control ion entry in the presence of salt in the medium (Hajibagheri et al., 1978). Werner and Finkelstein, (1995) also reported that high salt concentrations inhibited root and shoot elongation by slowing the plant's water uptake.

Table 6. Averages and groups of root and shoot length traits

Genotypes	RL NaCl Levels					SL NaCl Levels				
	0	75	150	225	Mean	0	75	150	225	Mean
G1	21.10s-u	24.57p-r	35.74ı-k	19.09u-w	25.12F	78.28ef	49.19lm	24.84r-t	12.83w	41.28H
G2	19.53u-w	27.12o-p	30.29mn	19.15u-w	24.02F	86.83d	69.214h-ı	37.11o	23.75st	54.23C
G3	17.049vw	25.04o-r	37.76h-k	32.57lm	28.10E	85.51d	56.39k	43.54n	28.14q-r	53.39CD
G4	58.79a	38.57hı	43.13fg	27.88no	42.09A	94.75bc	74.15g	51.30g	18.01l	59.55B
G5	55.50b	23.83q-s	38.09h-j	19.51u-w	34.23C	97.73ı	66.57ı	37.99o	17.87v	55.04C
G6	43.347ef	36.37ı-k	46.33de	25.40o-r	37.86B	93.29c	76.16fg	51.28l	25.28rs	61.50AB
G7	51.41c	35.03j-l	42.44fg	24.54p-r	38.36B	107.35a	70.58h	46.37nb	26.09rs	62.60A
G8	39.98gh	27.04op	38.51hı	25.50o-r	32.76CD	85.11d	62.11j	33.52p	18.54uv	49.82F
G9	40.35f-h	30.40mn	22.69r-t	20.03t-v	28.36E	69.81hı	56.35k	28.32qr	19.40uv	43.47G
G10	47.63d	30.33mn	34.91kl	16.81w	32.42D	96.87b	62.11j	29.87q	12.80w	50.41EF
G11	36.22ı-k	35.55ı-l	37.61h-k	25.96o-q	33.84CD	80.90e	67.50hı	37.81o	21.78tu	52.00DE
Mean	39.17 A	30.35 C	37.04 B	23.31 D		88.77 A	64.57 B	38.36 C	20.41 D	

Table 7. Averages and groups of root and shoot fresh weight traits

Genotypes	RFW NaCl Levels					SFW NaCl Levels				
	0	75	150	225	Mean	0	75	150	225	Mean
G1	0.0635h-k	0.0655g-ı	0.0640g-j	0.0234s-u	0.0541A	0.4177d	0.3419h	0.2835lm	0.0749rs	0.2795E
G2	0.0553l-o	0.0545m-o	0.0334qr	0.019uv	0.0407E	0.4194d	0.3142ıj	0.1704p	0.1120q	0.2540F
G3	0.0869bc	0.0710e-g	0.0756d-f	0.0201t-v	0.0634B	0.5861a	0.3779fg	0.2932kl	0.0797r	0.3342C
G4	0.0696f-h	0.0613ı-m	0.0658g-ı	0.0451p	0.0605B	0.4488c	0.3078ı-k	0.2917kl	0.1060q	0.2886E
G5	0.0889a-c	0.0620ı-l	0.0473p	0.0040x	0.0505CD	0.5350b	0.3884fg	0.2714m	0.0300t	0.3062D
G6	0.0774de	0.0669g-ı	0.0770de	0.0353q	0.0642B	0.4505c	0.4123de	0.3011j-l	0.4444c	0.4021A
G7	0.0940ab	0.0774de	0.0913ab	0.0277rs	0.0726A	0.5752a	0.4533c	0.3715g	0.0598s	0.3649B
G8	0.0581j-n	0.0634h-k	0.0564k-n	0.0169uv	0.0487D	0.3207ı	0.3449h	0.2238n	0.0707rs	0.2400G
G9	0.0945a	0.0826cd	0.0516n-p	0.0279rs	0.0641B	0.4234d	0.3955ef	0.2343n	0.1034q	0.2891E
G10	0.0484op	0.0355q	0.0461p	0.0148vw	0.0362F	0.3182ıj	0.2361n	0.1913o	0.0402t	0.1965H
G11	0.0267st	0.0344qr	0.0370q	0.0099wx	0.0270G	0.1770op	0.2167n	0.1772op	0.0629rs	0.1584I
Mean	0.0694 A	0.0613 B	0.0587 C	0.0222 D		0.4247 A	0.3444 B	0.2554 C	0.1076 D	

Table 8. Averages and groups of root and shoot dry weight traits

Genotypes	RDW NaCl Levels					SDW NaCl Levels				
	0	75	150	225	Mean	0	75	150	225	Mean
G1	0.0063h-j	0.0086bc	0.0069g	0.0028tu	0.0061B	0.0316de	0.0343cd	0.0273gh	0.0160mn	0.0273C
G2	0.0048mn	0.0052lm	0.0039qr	0.0025uv	0.0041E	0.0274gh	0.0277g	0.0184k-m	0.0169lm	0.0226E
G3	0.0084bc	0.0082cd	0.0047n-p	0.0024uv	0.0059B	0.0280g	0.0404a	0.0370bc	0.0119o	0.0293B
G4	0.0071fg	0.0052l-n	0.0061ı-k	0.0031t	0.0053C	0.0277g	0.0245ı	0.0312ef	0.0212jk	0.0261D
G5	0.0058jk	0.0057kl	0.0068gh	0.0009x	0.0048D	0.0264g-ı	0.0291e-g	0.0287fg	0.0048q	0.0222E
G6	0.0057kl	0.0060ı-k	0.0064hı	0.0036rs	0.0054C	0.0266g-ı	0.0286fg	0.0317de	0.0207jk	0.0269CD
G7	0.0107a	0.0087b	0.0103a	0.0023uv	0.0080A	0.0352c	0.0388ab	0.0408a	0.0120o	0.0317A
G8	0.0044o-q	0.0059ı-k	0.0051mn	0.0020vw	0.0043E	0.0216j	0.0308ef	0.0248hı	0.0119o	0.0222E
G9	0.0079de	0.0075ef	0.0056kl	0.0028tu	0.0059B	0.0351c	0.0368bc	0.0285fg	0.0170lm	0.0293B
G10	0.0032st	0.0025uv	0.0042p-r	0.0024uv	0.0030F	0.0188j-l	0.0190j-l	0.0214j	0.0081p	0.0168F
G11	0.0024uv	0.0021v	0.0037r	0.0015w	0.0024G	0.0124o	0.0138no	0.0191j-l	0.0074pq	0.0132G
Mean	0.0060 A	0.0060 A	0.0058 B	0.0024 C		0.0264 C	0.0294 A	0.0280 B	0.0134 D	

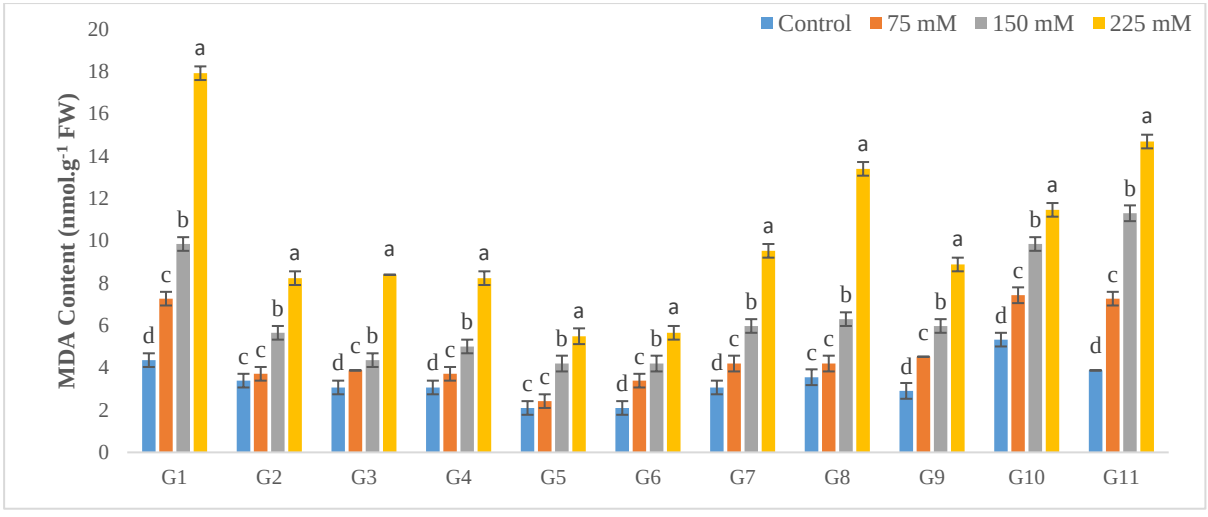
When the correlation values of the bilateral relations between the examined traits (Table 9) are examined; It was determined that there was a significant and positive relationship at the level of 1% between germination percentage and germination rate, seed viability index, root and shoot lengths and fresh and dry weights, normal seedlings and abnormal seedlings and a significant and negative relationship at the 1% level between the germination rate and the mean germination time. It was determined that there was a significant positive relationship at the level of 1% between the salinity tolerance index and shoot dry weight, a significant negative relationship at the level of 1% between shoot length and 5% between shoot fresh weight. It was determined that there was a significant and positive relationship at the level of 1% between normal seedling and germination percentage, germination rate, seed viability index, root and shoot lengths and fresh dry weights. In the study, it is thought that the different responses of flax varieties to NaCl concentrations may be due to cell wall or membrane permeability, NaCl toxicity and plasma membrane functions of the cultivars. (Tobe, Li and Omasa 2004). Significant and positive relations between the examined traits were found to contribute to growth and development due to salt stress (Koçak et al., 2022). They reported that seedling

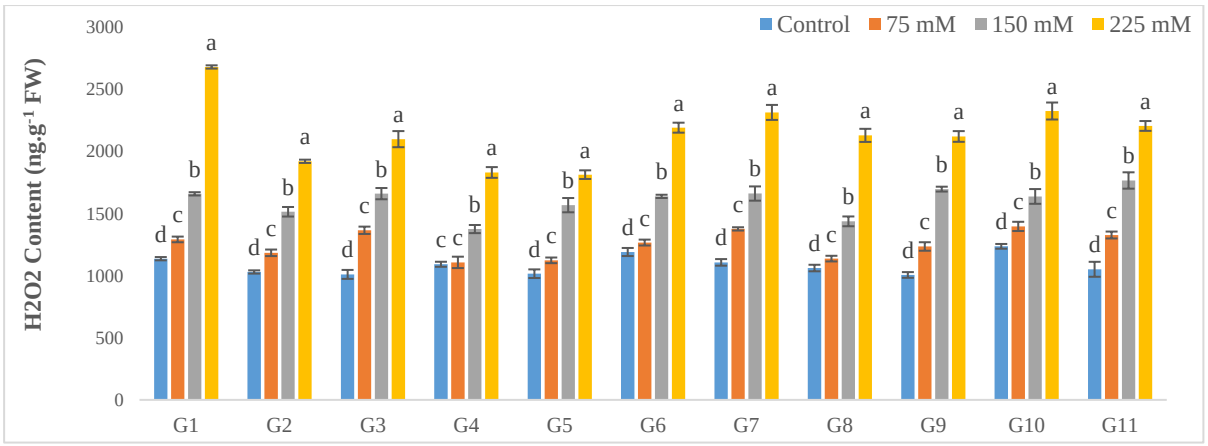
characteristics can be used as a valid parameter in the selection of genotypes that tolerate salinity stress better (Rajabi et al., 2020) and allow the selection of varieties that are resistant to salt stress in the early growth period of plants (Foti et al., 2019).

Table 9. Correlation values of the bilateral relations between the examined traits

Traits	GP	Rs	MGT	SVI	STI	RL	SL	RFW	SFW	RDW	SDW	NP
Rs	0.7925**											
MGT	0.1331	-0.3824**										
SVI	0.5872**	0.8582**	-0.4866**									
STI	-0.0034	-0.0751	0.1118	-0.2596**								
RL	-0.1335	0.229**	-0.5524**	0.4541**	-0.1402							
SL	-0.113	0.3215**	-0.7073**	0.644**	-0.4228**	0.5298**						
RFW	0.3877**	0.7152**	-0.5639**	0.8341**	-0.011	0.509**	0.6336**					
SFW	0.2465**	0.6191**	-0.6219**	0.7982**	-0.1808*	0.4193**	0.7872**	0.8589**				
RDW	0.4882**	0.729**	-0.4295**	0.7885**	0.106	0.3537**	0.4775**	0.8754**	0.7886**			
SDW	0.5025**	0.7109**	-0.4235**	0.7389**	0.2385**	0.3306**	0.4392**	0.8781**	0.7536**	0.8858**		
NP	0.5692**	0.7606**	-0.3758**	0.8251**	0.0144	0.3104**	0.5167**	0.8217**	0.758**	0.8446**	0.8564**	
ANP	0.5187**	0.1344	0.4784**	-0.1907*	0.0027	-0.4153**	-0.6345**	-0.3564**	-0.4485**	-0.2916**	-0.2648**	-0.3137**

***:%1; *: %5 statistically significant at the level*





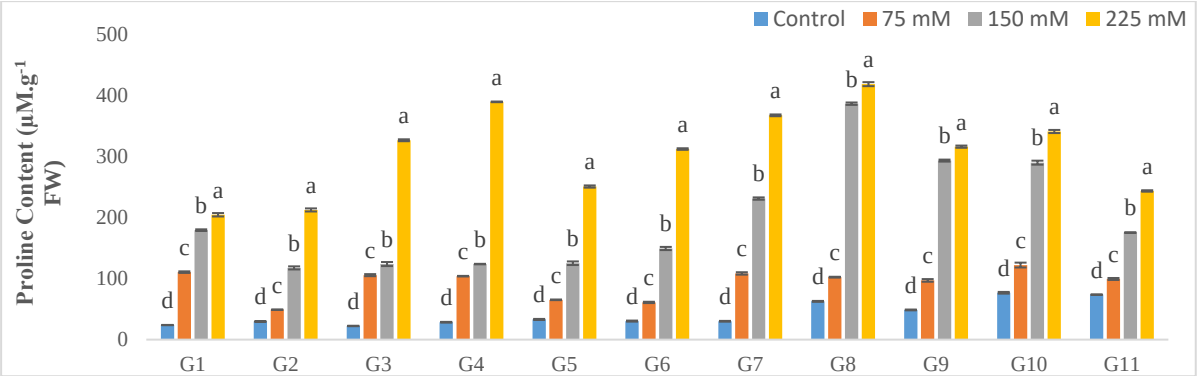


Figure 1. The effect of different salt concentrations on H2O2, MDA, and proline contents in eleven flax varieties.

When evaluated in terms of H2O2 content, it can be said that G1, G6, G7, and G10 resistant varieties, G2, G3, G5, and G9 sensitive varieties, G4, G8, and G11 varieties have moderate sensitivity. When evaluated in terms of MDA content, which is a measure of membrane damage, it can be said that G5, G6, and G7 resistant cultivars, G1, G8, and G11 susceptible cultivars, G2, G3, G4, G9, and G10 cultivars are moderately susceptible. When evaluated in terms of MDA content, which is a measure of membrane damage, it can be said that G1, G2, G5, and G11 resistant cultivars, G3 and G4 susceptible cultivars, G6, G7, G8, G9, and G10 cultivars are moderately susceptible.

Saglam et al. (2014) In a study conducted on different varieties of maize, it was reported that the hydrogen peroxide content of the resistant variety was significantly higher than the susceptible variety. They noted that proline levels increased significantly in parallel with the increase in salt concentration in three varieties of flax seedlings exposed to three different salt concentrations of 200, 400 and 600 mg/L (El-Bassiouny and Sadak, 2015). In a study conducted on five different flax varieties exposed to 100 mM NaCl stress, they stated that resistant flax varieties activate the antioxidant system and scavenge ROS, and this situation leads to a decrease in the Na/K ratio by protecting the membrane integrity (El-Beltagi et al. 2008). When the biochemical findings of eleven flax varieties used in the present study are evaluated as a whole; the data showed that the salt stress tolerance of G1, G7 and G6 cultivars are high, respectively; It indicates that the G9, G2 and G3 cultivars are also sensitive seedlings to salt stress. The remaining G4, G5, G8, G10 and G11 cultivars indicate that they are positioned between sensitive and resistant cultivars due to their similar responses to the severity of stress and the evaluations between parameters. Data from the present study were in agreement with some studies and diverged from others. It is thought that these differences are due to the genetic structure of the varieties, among the main reasons.

4. Conclusion

In this study; It was concluded that salt stress causes abnormalities in germination and early development of seeds in flax plants. It is thought that these abnormalities in the germination of seeds may negatively affect the development of the plant in later stages and cause a decrease in yield. It was determined that the most appropriate dose for the flax varieties used in the study was 75 mM NaCl and it had a germination promoting effect. However, it was concluded that 150 mM NaCl concentration promoted root length but negatively affected shoot length. According to the data obtained from the correlation analysis, it was determined that there was a significant and positive relationship between germination percentage and germination rate, seed viability index, root and shoot

lengths and fresh and dry weights, normal seedlings and abnormal seedlings. The contents of H2O2, MDA and proline, which are the most important biochemical parameters of salt stress, indicate that all the varieties examined continue to struggle against 150 mM salt stress, and metabolic activities are suppressed under 225 mM salt stress. When the anatomical, physiological and biochemical findings of the cultivars in the early development period are evaluated as a whole, the most sensitive cultivars to salinity are G2, G10 and G11 cultivars, moderately sensitive cultivars are G1, G4, G5, and G8 cultivars, and the most stable cultivar in terms of all doses is G3. G6, G7, and G9 varieties were found to show good results in terms of all traits. However, it has been concluded that it is necessary to carry out these cultivars under field conditions in order to obtain more decisive results.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

References

Apel, K., Hirt, H., 2004, Reactive oxygen species: metabolism, oxidative stress and signal transduction, *Annual Review of Plant Biology*, 55: 373-399.

Asada, K., 1999, The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons, *Annual Review of Plant Physiology and Plant Molecular Biology*, 50: 601-39.

Ashkan, A., Jalal, M. 2013. Effects of salinity stress on seed germination and seedling vigor indices of two halophytic plant species (Agropyron elongatum and A. pectiniforme). *International Journal of Agriculture and Crop Sciences*, 5(22): 2669-2676.

Ashraf, M., 2004. Some important physiological selection criteria for salt tolerance in plants. *Flora-Morphology, Distribution, Functional Ecology of Plants*, 199(5): 361-376.

Assaha, D.V., Ueda, A., Saneoka, H., Al-Yahyai, R., Yaish, M.W., 2017. The role of Na+ and K+ transporters in salt stress adaptation in glycophytes. *Frontiers in physiology*, 8: 509.

Bates, L., Waldren, R., Teare, I., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39:189–198.

Bilmez-Özçınar, A., 2021, Yağlı tohumlu bitkilerin tuzluluğa toleransı, Kalkınma Odaklı Doğal Kaynak Temelli İnovasyon, Iksad Publications, Ankara, 27-40.

Chuamnakthong, S., Nampei, M., Ueda, A., 2019. Characterization of Na+ exclusion mechanism in rice under saline-alkaline stress conditions. *Plant Science*, 287: 110171.

Datta, K.S., Dayal, J., 1991. Studies on germination and early seedling growth of gram (*Cicer arietinum* L.) as affected by salinity. *New Trends in Plant Physiology*, 1: 273-276.

El-Bassiouny, H., Sadak, M.S., 2015. Impact of foliar application of ascorbic acid and α-tocopherol on antioxidant activity and some biochemical aspects of flax cultivars under salinity stress. *Acta Biológica Colombiana*, 20(2): 209-222.

El-Beltagi, H.S., Salama, Z.A., El Hariri, D. M., 2008. Some biochemical markers for evaluation of flax cultivars under salt stress conditions. *Journal of Natural Fibers*, 5(4): 316-330.

Ellis, R.H., Roberts, E.H., 1981. The quantification of ageing and survival in orthodox seeds. Seed Science and Technology (Netherlands).

FAOSTAT, 2020. Dünyada keten tohumu üretimi. https://www.fao.org/faostat/en/#data/QCL/visualize (Erişim tarihi: 10.12.2022).

Fang, S., Wang, J., Wei, Z., Zhu, Z., 2006. Methods to break seed dormancy in Cyclocarya paliurus (Batal) Iljinskaja. *Scientia Horticulturae*, 110(3): 305-309.

Foti, C., Khah, E.M., Pavli, O.I., 2019. Germination profiling of lentil genotypes subjected to salinity stress. *Plant Biology*, 21(3): 480-486.

Hall, L.M., Booker, H., Siloto, R.M., Jhala, A.J., Weselake, R.J., 2016. Flax (*Linum usitatissimum* L.). In Industrial oil crops (pp. 157-194). AOCS Press.

Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of biochemistry and biophysics*, 125(1): 189-198.

Houle, G., Morel, L., Reynolds, C.E., Siégel, J., 2001. The effect of salinity on different developmental stages of an endemic annual plant, Aster laurentianus (*Asteraceae*). *American Journal of Botany*, 88(1): 62-67.

Huang, J., Redmann, R.E., 1995. Salt tolerance of *Hordeum* and *Brassica* species during germination and early seedling growth. *Canadian Journal of Plant Science*, 75(4): 815-819.

Iqbal, M., Ashraf, M., Jamil, A., Ur-Rehman, S., 2006. Does seed priming induce changes in the levels of some endogenous plant hormones in hexaploid wheat plants under salt stress? *Journal of integrative plant Biology*, 48(2): 181-189.

Jaleel, C.A., Gopi, R., Sankar, B., Manivannan, P., Kishorekumar, A., Sridharan, R., Panneerselvam, R., 2007. Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in Catharanthus roseus seedlings under salt stress. *South African Journal of Botany*, 73(2): 190-195.

Khayamim, S., Tavkol Afshari, R., Sadeghian, S.Y., Poustini, K., Roozbeh, F., Abbasi, Z., 2014. Seed germination, plant establishment, and yield of sugar beet genotypes under salinity stress. *Journal of Agricultural Science and Technology*, 16(4): 779-790.

Koçak, M.Z., Göre, M., Kurt, O., 2022. The effect of different salinity levels on germination development of some flax (*Linum usitatissimum* L.) varieties. *Turkish Journal of Agriculture-Food Science and Technology*, 10(4): 657-662.

Lay, C.L., Dybing, C.D., Robbelen, G., Downey, R.K., Ashri, A., 1990. Oil crops of the world. McGraw-Hill.

Li, J., Yin, L.Y., Jongsma, M.A., Wang, C. Y., 2011. Effects of light, hydropriming and abiotic stress on seed germination, and shoot and root growth of pyrethrum (*Tanacetum cinerariifolium*). *Industrial crops and products*, 34(3): 1543-1549.

Maas, E.V., Nieman, R.H., 1978. Physiology of plant tolerance to salinity. *Crop Tolerance To Suboptimal Land Conditions*, 32: 277-299.

Misra, N., Dwivedi, U. N., 2004. Genotypic difference in salinity tolerance of green gram cultivars. *Plant Science*, 166(5): 1135-1142.

Moghaddam, M., Babaei, K., Saeedi Pooya, E., 2018. Germination and growth response of flax (Linum usitatissimum) to salinity stress by different salt types and concentrations. *Journal of Plant Nutrition*, 41(5): 563-573.

Pan, J., Li, Z., Dai, S., Ding, H., Wang, Q., Li, X., Liu, W., 2020. Integrative analyses of transcriptomics and metabolomics upon seed germination of foxtail millet in response to salinity. *Scientific reports*, 10(1): 1-16.

Parida, A.K., Das, A.B., 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicology And Environmental Safety, 60: 324-349.

Rajabi Dehnavi, A., Zahedi, M., Ludwiczak, A., Cardenas Perez, S., Piernik, A., 2020. Effect of salinity on seed germination and seedling development of sorghum (*Sorghum bicolor* L.) Moench) genotypes. *Agronomy*, 10(6), 859.

Saglam, A., Kadioglu, A., Demiralay, M., Terzi, R., 2014. Leaf rolling reduces photosynthetic loss in maize under severe drought. *Acta Botanica Croatica*, 73(2): 315-323.

Shahid, S.A., Zaman, M., Heng, L., 2018. Introduction to soil salinity, sodicity and diagnostics techniques. In Guideline for salinity assessment, mitigation and adaptation using nuclear and related techniques (pp. 1-42). Springer, Cham.

Shrivastava, P., Kumar, R., 2015. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi journal of biological sciences*, 22(2): 123-131.

Song, J., Chen, M., Feng, G., Jia, Y., Wang, B., Zhang, F., 2009. Effect of salinity on growth, ion accumulation and the roles of ions in osmotic adjustment of two populations of Suaeda salsa. *Plant and Soil*, 314(1): 133-141.

Tobe, K., Li, X., Omasa, K., 2004. Effects of five different salts on seed germination and seedling growth of Haloxylon ammodendron (*Chenopodiaceae*). *Seed Science Research*, 14(4): 345-353.

Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants, protective role of exogenous polyamines. *Plant Sciences* 151:59–66.

Vicente, O., Boscaiu, M., Naranjo, M.Á., Estrelles, E., Bellés, J.M., Soriano, P., 2004. Responses to salt stress in the halophyte Plantago crassifolia (Plantaginaceae). *Journal of Arid Environments*, 58(4): 463-481.

Werner, J.E., Finkelstein, R.R., 1995. Arabidopsis mutants with reduced response to NaCl and osmotic stress. *Physiologia Plantarum*, 93(4): 659-666.

Yamaguchi, T., Blumwald, E., 2005. Developing salt-tolerant crop plants: challenges and opportunities. *Trends in plant science*, 10(12): 615-620.

Yang, F., Chen, H., Liu, C., Li, L., Liu, L., Han, X., Sha, A., 2020. Transcriptome profile analysis of two Vicia faba cultivars with contrasting salinity tolerance during seed germination. *Scientific reports*, 10(1): 1-10.

Yokoi, S., Bressan, R.A. Hasegawa, P.M., 2002. Salt stress tolerance of plants, JIRCAS Working Report, 25-33.

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