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ASSESSMENT OF SALT STRESS RESISTANCE OF COTTON VARIETIES BASED ON DIFFERENT PARAMETERS

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Abstract. Salinity is one of the serious threats to cotton growth and productivity. The presence of a wide genetic variability of genotypes in germplasm may be an advantage in the creation of salt-resistant varieties in future breeding programs. In this study were analyzed the resistance to salt (NaCl) stress of 48 genotypes belonging to the species *G. hirsutum* L. and *G. barbedense* L., as well as chromosome substitution lines. The effect of different concentrations of NaCl on different parameters of 48 cotton genotypes were evaluated and cluster analysis was performed. The correlation between the concentration of salt and the value of different parameters were studied and the lowest germination rate for all genotypes was observed at 200 mM NaCl. There were found a negative correlation between salt concentration and FGP, CVG, GRI, GI and a positive correlation with MGT. According to results of analysos genotypes Navai-9, Karabakh-11, Agdash-3, Flash, AP-317, Kyrgyzstan-174, Karabakh-12, Tashkent-2 and chromosome substitution lines CSB-11, CSB-14, CSB-05, CSB-17 had the highest germination index and showed high resistance to salt stress.

Keywords: *cotton, salinity, germination index, germination rate index, mean germination time.*

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Abbreviations:

FGP-final germination percentage;
CVG-coefficient velocity of germination;
GRI-germination rate index;
GI-germination index;
MGT- mean germination time.

1. Introduction

Cotton is a key agricultural product for textile fiber and the world's second-largest oil seed crop. (Munawar *et al.* 2021). As a primary agriculture crop that covered the biggest textile manufacturing industries it devising a strong yearly influence on country economic value of $600 billion all over the world (Jabran *et al.*, 2019; Munawar *et al.* 2021).

Cotton yield is strongly influenced by several biotic and abiotic factors, resulting in significant losses in target yield. Salinization occurs due to the accumulation of high concentrations of salt in soil and water systems. (Manikandan *et al.* 2019). Soil salinization is rapidly increasing day by day and became a global environmental

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problem (Ahammed *et al*. 2018; Shakeel & Hasanuzzaman, 2020). Salt accumulation in the soil inhibits seed germination and plant growth and creates osmotic imbalances and toxicity, resulting in poor plant establishment (Ahmad *et al.* 2002; Bednarz *et al.* 2002; Shakeel & Hasanuzzaman, 2020).

While some plants can germinate at high NaCl concentrations, others are more sensitive during germination. The effect of salinity on plant growth and development is related to the stage of plant development (Chartzoulakis & Loupassaki, 1997). Although salinity stress affects all stages of plant growth and development, depending on the species of plant, germination or other specific developmental stage may be the most critical stage for salinity stress (Khoshsokan *et al.* 2012). This stress also leads to degradation of photosynthetic pigments, destruction of chloroplasts, reduction of chlorophyll fluorescence, and reduction of net photosynthetic ratios (Lluch *et al.* 2007; Chaum *et al*. 2009).

Cotton seed germination is an important stage, it is also one of the most vulnerable to severe climate conditions. Salinity can prevent seed germination by limiting the plant's ability to absorb water, causing dehydration, and depriving it of nutrients by disrupting the ions uptake mechanism (Wang *et al.* 2011; Munawar *et al.* 2021). As the most important natural textile fibre crop in the world, cotton is also the second most inherently salt-tolerant crop, having a 7.7 dS/m threshold level. (Huang *et al.* 2013; Qishen *et al.* 2021). Different cotton species, and even different cultivars within a species, have varying salt tolerance abilities. (Sun *et al.* 2018; Qishen *et al.* 2021). High levels of salt ultimately inhibit cotton growth and reduce productivity, especially when exposure occurs at the germination or seedling stage (Qishen *et al.* 2021**)**. Effects of salt (NaCl) on cotton differ in accordance with the change in salt concentration, depending upon the time period of salt exposure and growth stage in which the plant is exposed to stress. Cotton is considered a salt-tolerant crop after barley, but its yield decreases by 5% per unit dS/m as the stress limit increases. (Chinnusamy *et al.* 2005, Munawar *et al.* 2021). Under field conditions, 20 dS/m stress treatment would result in a yield reduction of approximately 60%. (Higbie *et al.* 2010, Munawar *et al.* 2021). At 200 mM NaCl concentration, commercial cultivars of G. hirsutum exhibited poor and delayed germination. (Javid *et al.* 2001, Ahmad *et al.* 2002). Although G. arboretum has been reported to be sensitive to NaCl, filter paper studies of G. arboreum cotton varieties seed germination was found to be moderately affected by salinity stress (Ashraf, 2002, Hassan *et al.* 2014, Manikandan *et al.* 2019). According to Wang *et al.* (2011), germination stage are more sensitive to salt stress than juvenile stage. In cotton, a sharp decrease in the percentage of germination was observed above 10 dSm^{-1}. In response to stress due to salinity, cotton also retards the germination and emergence stages (Khorsandi & Anagoli, 2009; Ma *et al*. 2011).

For this reason the main purpose of the research work is the study of salt resistance of local and introduced genotypes of cotton based on different parameters and screening of resistant genotypes.

2. Material and methods

Plant material

The material for the study were healthy seeds of 48 cotton genotypes. 13 Azerbaijan cultivars (Agdash-3, AP-317, Bayraqdar, Barakat, Ganja-110, Ganja-114, Ganja-160, Ganja-182, Ganja-195, Ganja-200, Karabakh-11, Karabakh-12, Zafar), 4

Greece cultivars (Assos, Cristina, Prime, Select), 4 Uzbekistan cultivars (Tashkent-1, Tashkent-2, Tashkent-3, Navai-9), 1 Kyrgyzstan cultivar (Kyrgyzstan-174), 9 Turkey cultivars (Beyazaltun-440, CSN-12, Edessa, Flash, Lima, May-344, PG, Sezener, Carisma) and 15 chromosome substitution lines (CSB-02, CSB-04, CSB-05, CSB-06, CSB-07, CSB-11, CSB-12, CSB-14, CSB-15, CSB-16, CSB-17, CSB-18, CSB-22, CSB-25, CSB-26, and their parents TM-1 (*G. hirsutum* L.) and Pima 3-79 *(G. Barbadense* L.) from USA were used as the material of the study.

The research was carried out in the department of Technical and Forage Plants of the Institute of Genetic Resources. 25 seed samples of each genotype were planted in Petri dishes in 4 replications. Salinity indicators of genotypes was calculated based on the percentage of final germination using controls and NaCl salt concentrations of 50 mM, 100 mM, 150 mM and 200 mM. For recording germinated seeds the Petri plates were kept for 10 days in a chamber and germinated seeds were counted every day during ten days.

Method

Different Germination Parameters were calculated by Al-Mudarris method (Kader, 2005) using the following formulas:

Final germination percentage (FGP in %) was calculated by the formula FGP = Ng/Nt × 100; Ng = Total number of seeds germinated; Nt = Total number of seeds evaluated

Coefficient of velocity of germination (CVG) was calculated as follows:

CVG=N1 + N2 + · · · + Nx/100 × N1T1 + · · · + NxTx

N=No. of seeds germinated each day, T=No. of days from seeding corresponding to N

Mean time germination (MTG in days) was calculated as follows:

MTG (in days) = ΣF*x/ΣF; f=Seeds germinated on day x

Germination rate index (GRI) was calculated as follows:

GRI = G1/1 + G2/2 + … + Gx/x

G1 = germination percentage at first day; G2 = germination percentage at second day; Gx/x= germination percentage at x day

Germination index (GI) was calculated as follows:

GI = (10 × N1) + (9 × N2) + ... + (1 × N10)

N1, N2 and … are the number of germinated seeds by day

Numbers 10, 9,... are respectively the weights imposed on the number of seeds germinated at first day, second and other days.

Time spread of germination (TSG) was calculated as follows:

TSG = LDG (last day of germination) – FDG (first day of germination)

Analysis of germination parameters of genotypes was carried out using the IBM SPSS (SPSS Statistics version 26) software. Correlation between germination parameters were calculated for all 48 genotypes.

3. Results and discussion

Various methods have been developed to determine the resistance of plants to stres factors, one of which is to determine their resistance to salt stress during the initial development of plants. Germination potential, germination rate, fresh weight and viability index can be used to assess salt tolerance during the germination stage. It was reported seed germination is a good indicator of cotton salt tolerance (Ashraf, 2002).

Based on the results of the study, it was determined that the increase in salt concentration caused a decrease in the final germination percentage for all studied genotypes. According to the control, the relative final germination percentage at 50 mM salt concentration decreased by 2-23%, at 100 mM salt concentration by 11-42%, at 150 mM salt concentration by 24-56%, and at 200 mM salt concentration by 35-71%.

MGT is a measure of the time required for a seed population to germinate. This indicator focuses on the day when the most germination occurs, and the lower this parameter, the faster the seed population germinates (Kader, 2005). Although increasing of this indicator was observed for all genotypes compared to the control, the MGT indicator increased to different degrees in different genotypes. Although a positive correlation was observed between the increase of salt stress and the change of MGT in 41 genotypes, clear dynamics were not determined in 7 genotypes. Thus, in genotypes CSN-12, Lima, Prime, Karabakh-12 and in lines CSB-02, CSB-18 at 100 mM, in lines CSB-17, CSB-26 at 200 mM a decrease was observed compared to the previous concentration.

The germination rate index indicator is a parameter that reflects the germination percentage on each day of the germination period, so the higher the germination percentage and the shorter the duration, the higher the GRI value (Kader, 2005). Since the increase in salt stress causes a decrease in the relative final germination percentage and an increase in the germination period, it was determined that there is a positive correlation between the cycle index salt concentration for all studied genotypes.

Although similar indicators were observed between the first day and the last day of germination in some genotypes at different salt concentrations in the conducted research, it was determined that there was a wide diversity between the first and last days of germination for all genotypes with an increase in salt concentration compared to the control. Thus, in only 2 genotypes, the first germination occurred on the same day in the control and all salt concentrations, and in 46 genotypes, the first germination day in the control and 200 mM salt concentrations began on different days. Also, in 22 genotypes, germination in the control started before all salt concentrations, and in 26 genotypes, different dynamics were determined between the control and the first germination at different salt concentrations. Based on the analysis results of the last day of germination, this indicator increased in 35 genotypes compared to the control. In 7 genotypes, control and 50 mM salt concentration, in 1 genotype in control and 100 mM salt concentration, and in 5 genotypes in control, 50 mM and 100 mM salt concentration, this indicator overlapped. Although similar results were obtained for the TSG parameter in the control and some low salt concentrations, in general, it was determined that the increase in salt concentration caused a change in this parameter.

CVG is an indicator of germination rate, which increases when the number of germinated seeds increases and the time required for germination decreases. In theory, the highest possible CVG is 100. This can happen if all the seeds germinate on the first day. During the conducted research, a clear negative correlation was observed in the CVG indicator for all genotypes with the increase of salinity except for CSB-02, CSB-17, CSB-18, CSB-26, CSN-12, Edessa, Lima Karabakh-12 genotypes.

The use of germination data analysis methods is prone to misinterpretation if germination percentage, rate, spread are not taken into account in one measurement. It has been determined that GI is an important parameter for accurate evaluation in the context of the tested parameters (Kader, 2005). In GI, seeds that germinate on the first day are given maximum value and those that germinate later are given less value. This

indicator increases the difference between cotton seeds through an easily comparable numerical measurement. Based on the cluster analysis of the germination index of cotton genotypes of different origins at 200 mM salt concentration, the genotypes were grouped into 5 main groups (Figure 1).

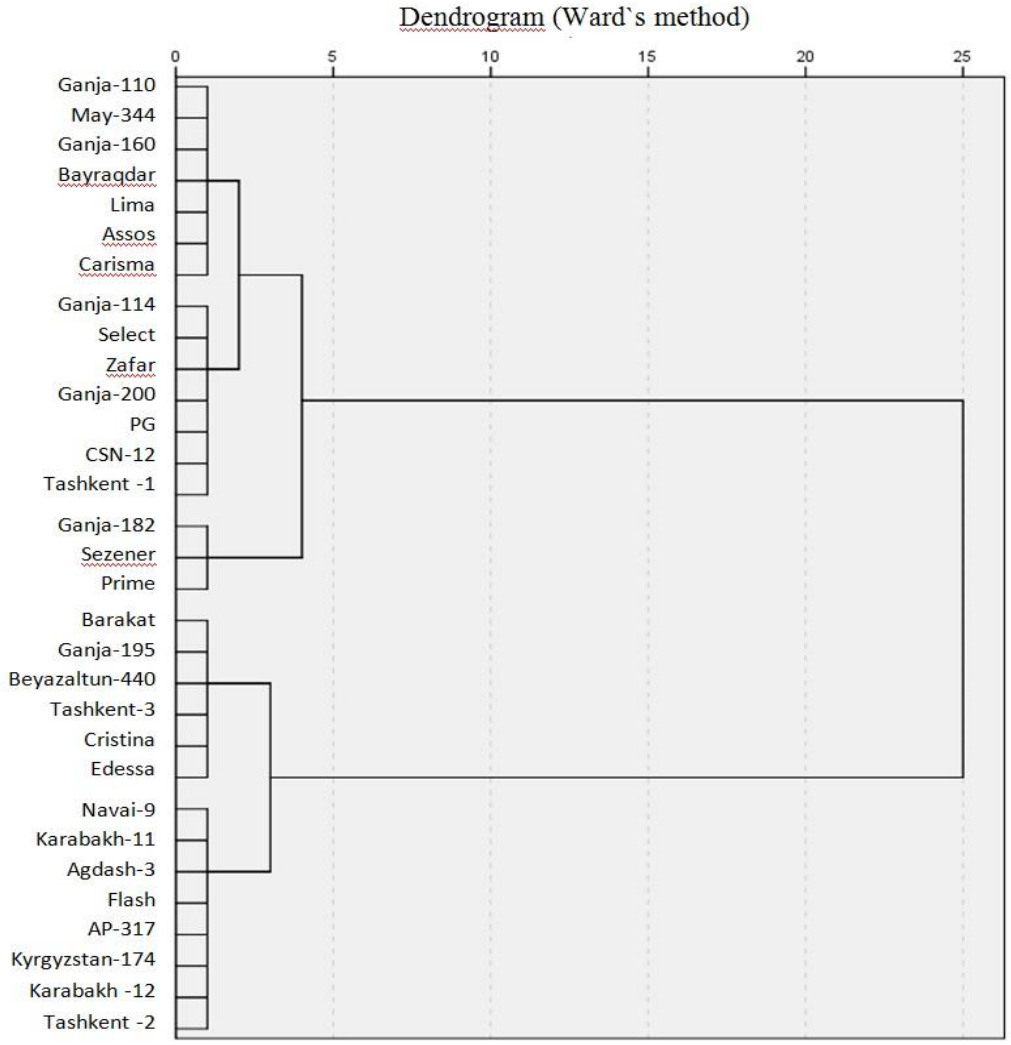

Fig. 1. Cluster analysis of cotton genotypes based on germination index at 200 mM salt concentration

The germination index of Ganja-110, May-344, Ganja-160, Bayraqdar, Lima, Assos, Carisma genotypes, collected in the first group, varied between 163-193 and these genotypes were evaluated as sensitive. The germination index of Ganja-114, Select, Zafar, Ganja-200, PG, CSN-12, Tashkent-1 genotypes included in the second group varied between 204-254 and this group was moderately resistant to salt stress. Genotypes Ganja-182, Sezener and Prime were collected in group III and the lowest germination index was observed in these genotypes and they were evaluated as highly sensitive genotypes. The germination index of group IV, which includes Baraket, Ganja-195, Beyazaltun-440, Tashkent-3, Cristina, and Edessa genotypes, varied between 274-310 and was evaluated as a salt-resistant group. Genotypes included in group V (Navai-9, Karabakh-11, Agdash-3, Flash, AP-317, Kyrgyzstan-174, Karabakh-

12, Tashkent-2) had the highest germination index (340-403) and showed high resistance to salt stress.

According to the cluster analysis of the germination index of chromosome substitution lines and their parents at 200 mM salt concentration, the genotypes were grouped into 3 main groups (Figure 2).

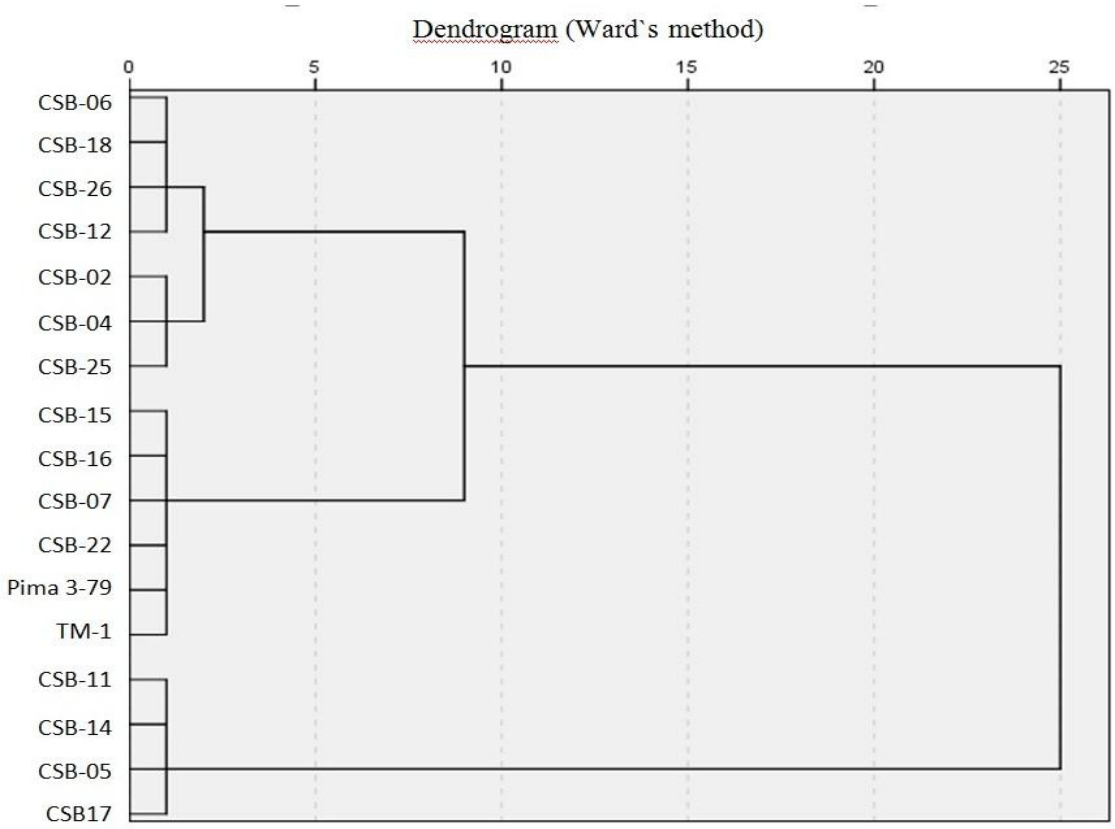

Fig. 2. Cluster analysis of chromosome substitution lines and their parents based on germination index at 200 mM salt concentration

The lowest value of the GI index was in lines CSB-06, CSB-18, CSB-26, CSB-12, CSB-02, CSB-04, CSB-25 (106-182) and this group was evaluated as sensitive. The lines CSB-07, CSB-15, CSB-16, CSB-22 and parents TM-1 and Pima 3-79 were combined in second group. The GI indicators of these genotypes varied between 200-251 and were evaluated as a moderately salt-resistant group. The combined group of lines CSB-11, CSB-14, CSB-05 and CSB-17 had the highest GI index, ranging from 303-345 and was evaluated as resistant genotypes.

Regression analysis of data at normal and salt stress conditions was described in Table 1. According to result, there were significant regression between all evaluated genotypes and a significant difference of 99% was found between the increase of salt stress and germination parameters. Under salt stress condition all traits were significant at 1% probability level. This shows that the increase of salt stress caused the change of germination parameters.

The evaluation of the effect of salinity and germination parameters of genotypes According to Pearson's correlation analysis is shown in Table 2. Based on the results of the correlation analysis, a 99% significant dependence was found between salt

concentration and germination parameters. As can be seen from the table, a negative correlation was found between salinity and all other parameters except MGT at the 99 percent probability level. The increase in salt concentration caused a significant decrease in these indicators. A positive correlation between MGT and salinity was determined at the 99 percent probability level. An increase in hardness led to an increase in the mean germination time.

Table 1. Regression analysis for different germination parameters under salt stress

Model	Sum of Squares	df	Mean Square	*F*
Regression	275.016	5	55.003	234.264**
Residual	34.984	149	.235	
	310.000	154		

a. Dependent Variable: Salt Concentration
b. Predictors: (Constant), GI, MGT, CVG, GRI, FGP
** significantly different at 0.01

Table 2. Pearson's correlation coefficient (r) for different germination parameters under salt condition

	FGP	CVG	MGT	GRI	GI	Salt Concentration
FGP	1	.846**	-.806**	.955**	.984**	-.911**
CVG		1	-.939**	.950**	.923**	-.878**
MGT			1	-.863**	-.882**	.813**
GRI				1	.985**	-.937**
GI					1	-.935**
Salt Concentration						1

** significantly different at 0.01

The results obtained as a result of the conducted research were similar to the results obtained by Fuller et al. (2012) in wheat genotypes at different concentrations of NaCl.

3. Conclusion

Various germination parameters in cotton genotypes were significantly changed under salt stress. In the conducted research, germination parameters of 48 cotton genotypes were comparatively analyzed and cluster analysis was performed based on the complex indicator GI. Based on the results of the analysis, Agdash-3, AP-317, Karabakh-11, Karabakh-12, Navai-9, Flash, Kyrgyzstan-174, Tashkent-2 genotypes had the highest germination index and were evaluated as salt-resistant genotypes. The genotypes of the lines with the chromosome replaced had the highest indicators. Also, chromosome substitution lines CSB-11, CSB-14, CSB-05 and CSB-17 had the highest GI index and was evaluated as a resistant to the salt stress.

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