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Assessment of salt tolerance of wheat and maize genotypes according to physiological and yield indicators

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Abstract
To create new salt-tolerant varieties of wheat and maize, a comparative analysis of the physiological parameters of the parental and hybrid plants was carried out. Physiological parameters such as chlorophyll content, relative water content, and PSII activity have been studied in parental forms and hybrids. Based on the physiological indices determined during the grain filling stage, the highest tolerance was manifested by the parental forms, Garabagh, Gobustan, and the hybrids, Garabagh x Gobustan, Garabagh x Mirbashir, Garabagh x Sharg. Maize varieties Gurur, Zagatala- 68 and hybrid "Gurur x Zagatala -68" were resistant to 200 mmol salt concentration.

Keywords: hybrid, genotype, chlorophyll, activity, photosystem II, carotenoid

Introduction

Salinity is one of the abiotic stress factors decreasing plant productivity. The salinization of soils over time is particularly dangerous. The limitation of agricultural and fertile lands is an obstacle to meeting the food requirements of the population [Khan *et al*., 2010] [1]. In particular, the rapid growth of the population and the need in ensuring food security make more urgent the development of salt-tolerant varieties capable to grow in saline soils, and their extensive use. According to rough estimates, 521,700 hectares of plains in the Azerbaijan Republic were in a saline state in 2002 [Azizov, 2002] [2]. In 2007, this parameter increased to 661.9 thousand hectares and accounted for 46.6% of the land [Mammadov, 2007] [3].

One of the most effective measures taken to achieve high productivity under stress is the development of plants capable to adapt to salinity. The expression of genes regulating stress tolerance increases under high salt concentrations and ensures salt tolerance of plants [Garratt *et al*., 2002] [4].

According to some authors, developing more plastic wheat varieties, suitable for the regions of the republic is required because of the disturbance of ecological balance and the presence of abiotic stress factors. Therefore, stress tolerance in plant breeding is of great importance [Rustamov, Talai., 2017] [5]. Currently, in our country, extensive research has been carried out on salt-tolerance of local wheat varieties developed under the leadership of academician J.A. Aliyev as well as brought from abroad [Huseynova *et al*., 2008] [8].

Thus, numerous studies conducted in the world and in our country showed the perspectives of the development of the wheat varieties adapted to salinity.

Materials and Methods

The objects of the study were parental and hybrid wheat forms grown under normal and salinity (0,98% NaCl) conditions. The objects of the study were the genotypes of wheat Gobustan, Mirbashir-128, Barakatli-95, Garabagh, Gyrmyzygul, Sharg and their generation hybrids Garabagh x Gobustan, Gobustan x Barakatli-95, Gobustan x Gyrmyzygul, Barakatli 95 x Gobustan, Gobustan x Garabagh, Garabagh x Mirbashir, Garabagh x Sharg.

The second generation (F_2) hybrids obtained from seven combinations during the study were planted on the field of $1m^2$, under normal conditions, on October 25, 2019..For the experiments, 300g NaCl was added to the soil before sowing during tillering, earing, and grain-filling stages. Watering was performed at the tillering, earing, and grain-filling stages. Agrotechnical care was conducted in the experimental field. Samples were taken at the grain filling stage of the vegetation. Measurements were done *in vivo* and *in vitro* for the comparative study of the changes in physiological parameters caused by the salt effect.

The object of research was also the genotypes of maize Zagatala 420, Zagatala 514, Zagatala 68, Gurur and first generation F_1 hybrid Zagatala 68 x Gurur. Plant seeds germinated under laboratory conditions in Petri dishes and pots with soil using 150 mmol of sodium chloride solution. Germination energy was determined by counting three days old seedlings, and seed germination ability by counting seven days old seedlings as a percentage. In two-week old seedlings determined the content of photosynthetic pigments and the activity of photosystem II.

0.1 g of leaf samples of hybrid and parental forms taken from plants grown under both normal and saline conditions were homogenized using a pestle and mortar in 96% alcohol by adding $CaCO_3$, centrifuged at 200g, and a pure extract of chlorophyll pigments was obtained. The optical density of a solution of chlorophyll in alcohol was measured on an SP-2000 spectrophotometer at 665, 649, 440 nm, and the amounts of chlorophyll and carotenoids were determined [Wintermans, De Mots, 1965] [7]. The activity of PSII was established based on Fv/Fm using a photosynthesis analyzer (PAM Germany).

$F_v = F_m - F_0$ Where F_0 - fluorescence of leaves illuminated after dark treatment, F_m - fluorescence of light-saturated leaves.Water loss in leaves (RWC) was determined based on the method of Tambussi and colleagues [Tambussi E. A., Noges S, 2005] [8].

Results

To develop new, pure lines, salt tolerance of hybrids and parental forms was assessed based on physiological indices in the grain filling stage of the vegetation. When studying salt-tolerance of hybrids and parental forms, differences were detected in the relative amounts of chlorophyll a, chlorophyll b, carotenoids, RWC, as well as the photochemical activity of chloroplasts. The effect of salt on the amount of chlorophyll a, chlorophyll b, and carotenoids, which are the main physiological indicators, is manifested in different ways in both hybrids and parental forms. Based on the changes in the amount of pigments under salinity, the parental forms Garabagh, Gobustan, and Sharg were more tolerant. Thus, at least a 10% -decrease in the amount of chlorophyll a and chlorophyll b pigments due to the effect of salt was observed in these parental genotypes. Whereas, the most growth in carotenoids amounted to 4-7 % in these varieties. This process can be attributed to the increase in the protective function of carotenoids under salt stress. Based on RWC values, Garabagh and Gobustan varieties had the highest water content. Water loss in these varieties did not decrease but increased by 3%. The results obtained are presented in Table 1 and Table 2.

Based on the amounts of chlorophyll a and chlorophyll b, the hybrids, Garabagh x Gobustan, Garabagh x Mirbashir, Garabagh x Sharg are more tolerant to salinity. An increase in the amount of carotenoids was also observed in these hybrids. The highest values for RWC were found in the hybrid forms, Garabagh x Gobustan, Barakatli 95 x Gobustan, Garabagh x Sharg. RWC was 10% higher in these hybrids compared to others under stress. A 20% -decrease in RWC was observed in the hybrid form, Gobustan x Gyrmyzygul.

The first indicator of fluorescence in varieties grown in a saline environment is light energy. Energy loss occurs under salinity. In this case, the activity of the photosystem is reduced, which leads to the weakening development of the plant.

Based on the PS II activity, the parental forms, Garabagh, Gobustan, and the hybrids, Gobustan x Barakatli-95, Gobustan x Garabagh, and Gobustan x Sharg are more salt-tolerant (Figure.).

One of the most useful indicators of wheat is grain yield. High salt concentrations contributed to a decrease in grain yield. Among the genotypes, according to this indicator, the most salt tolerant were the hybrids Barakatli-95 x Gobustan and Garabakh x Mirbashir-128 (Table 3).

It revealed that under the influence of salt, germination and germination energy of maize seeds reduced. With the increase of sodium chloride concentration decreased the photochemical activity of chloroplasts and the content of photosynthetic pigments in seedlings. Differences between maize cultivars in the content of chlorophylls, carotenoids and in the parameters of fluorescence induction of leaves under salt action were revealed. (Table4).

As seen from the table, with increasing concentration, the percentage of seed germination, the content of chlorophyll and carotenoids, and the activity of photosystem II decreased. At a salt concentration of 150 mmol the pigment content and the activity of chloroplasts in the genotypes Zagatala 420 and Zagatala 514, while in varieties Gurur and Zagatala 68 remains at the control level. At a salt concentration of 200 mmol, the activity of photosystem II of genotypes Zagatala 420 and Zagatala 514 decreases to a greater extent than that of varieties Gurur and Zagatala 68.

Table 1: Physiological indices (photosynthetic pigments, RWC) of the parental and hybrid forms under normal conditions (mg/g fresh weight)

Parental forms (control)	Chla	Chlb	Chl(a+b)	Chl a/b	Carotenoids	RWC, %
1.Gobustan	2.93	0.53	3.47	5.44	1.04	70.9
2.Mirbashir -128	2.60	0.27	2.95	9.56	1.03	75.5
3.Barakatli -95	2.99	0.30	3.29	9.75	1.08	71.9
4.Garabagh	2.23	0.32	2.55	6.95	1.05	89.5
5. Gyrmyzygul -1	3.14	0.42	3.57	7.40	0.87	88.9
6. Sharg	2.89	0.48	3.38	5.93	1.17	70.5
F_2 hybrids (control)						
1. Garabagh xGobustan	2.43	0.54	2.98	4.44	1.17	67.9
2.Gobustan x Barakatli -9	2.63	0.30	2.93	8.70	1.04	98.6
3.Gobustan xGyrmyzygul-1	2.37	0.17	2.54	13.5	0.86	75.5
4. Barakatli -95 x Gobustan	3.07	0.26	3.33	11.78	1.35	67.4
5. Gobustan x Garabagh	1.80	0.28	2.09	6.34	0.60	92.3
6.Garabagh xMirbashir-128	1.89	0.37	2.26	5.10	0.57	94.8
7.Garabagh x Sharg	3.10	0.44	3.55	6.93	1.72	74.4

* Each value represents the mean ± SD (standard deviation) for the mean n = 3 independent experimets p=0.05

Table 2: Physiological indices (photosynthetic pigments, RWC) of the parental and hybrid forms of wheat under 1% NaCl (mg/g fresh weight)

Parental forms exposed to salt	Chla	Chlb	Chl(a+b)	Chl a/b	Carotenoids	RWC, %
1.Gobustan	1.94	0.48	2.42	4.04	0.54	73.1
2.Mirbashir-128	1.68	0.35	2.03	4.76	0.95	74.9
3.Barakatli -95	1.34	0.27	1.61	4.49	0.97	74.2
4.Garabagh	2.28	0.43	2.71	5.53	0.69	83.6
5.Gyrmyzygul -1	1.22	0.34	1.56	3.35	0.74	80.8
6.Sharg	1.23	0.26	1.50	4.47	0.38	76.9
F2 hybrids exposed to salt						
1.Garabagh x Gobustan	1.23	0.26	1.50	4.70	0.51	84.1
2.Gobustan xBarakatli-95	2.25	0.24	2.49	9.24	0.90	72.8
3.GobustanxGyrmyzygul-1	1.99	0.24	2.24	7.23	1.06	79.6
4.Barakatli -95 x Gobustan	1.24	0.27	1.51	4.58	0.62	81.8
5. Gobustanx Garabagh	1.34	0.28	1.63	4.65	0.63	75.6
6.Garabagh xMirbashir128	1.46	0.29	1.75	5.01	0.29	79.5
7.Garabagh xSharg	1.21	0.21	1.42	6.93	0.33	83.5

* Each value represents the mean ± SD (standard deviation) for the mean n = 3 independent experimets p=0.05

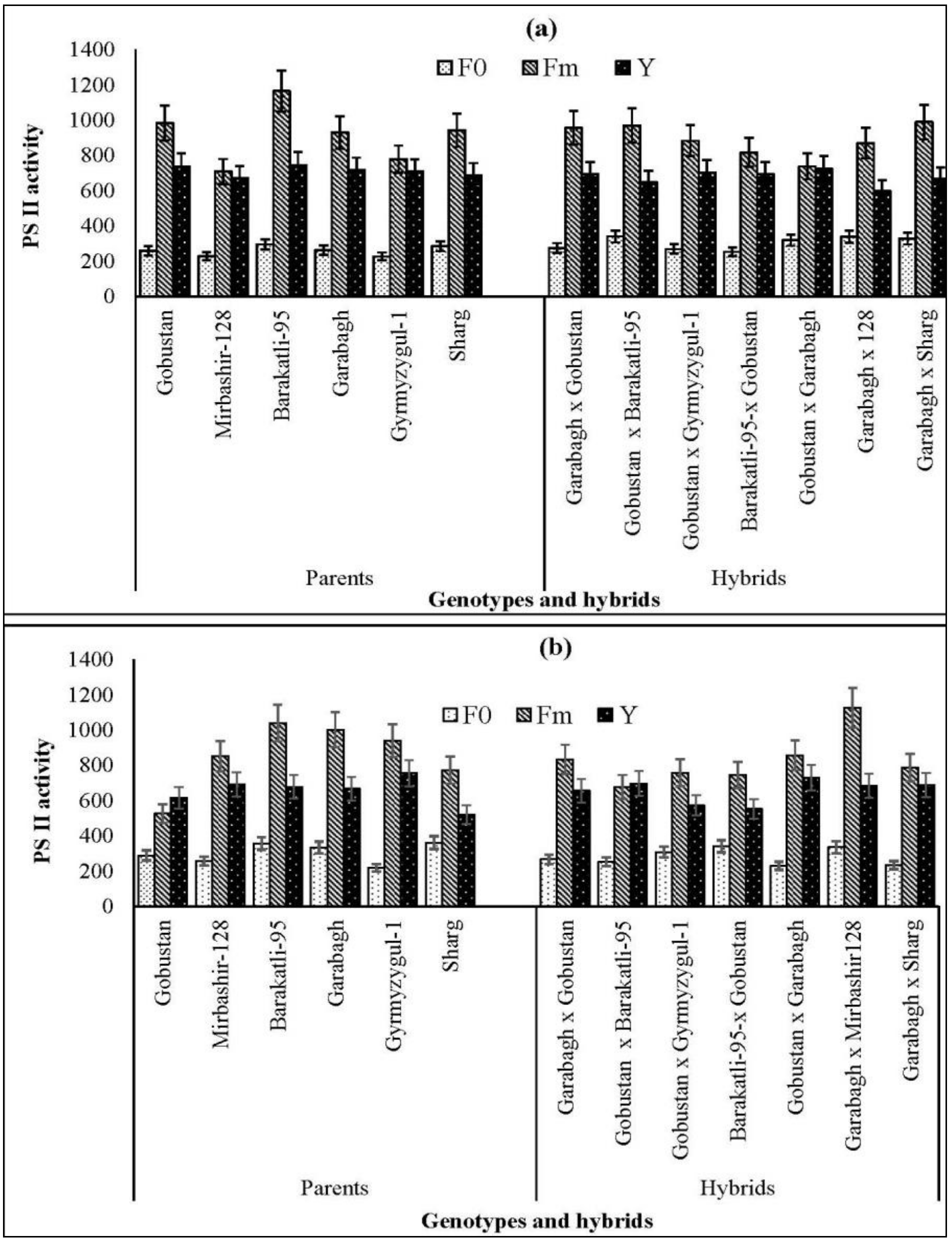

Fig 1: Effect of NaCl (0.98%) on the photochemical activity of the parental and hybrid forms during the grain filling stage A-control B- salt exposure

Table 3: Influence of sodium chloride (0,98%) on physiological and biochemical characteristics of maize varieties seedlings (mg/ g weight)

No	Varities	Germination, (%)	Cl (a+b)	Carotenoids	F_v / F_m	RWC, %	MDA, µmol/g	Sugars
1	Zagatala improved Control Salt	90±2 84±3	9.8±1.2 4.9±1.4	2.4±0.2 1.6±0.1	0.69 0.54	91 74	0.011 0.016	7.8±0.4 12.6±0.5
2	Zagatala68 Control Salt	88±2 70±1	10.7±1.9 6.4±1.2	2.7±0.3 2.1±0.1	0.78 0.75	90 82	0.015 0.021	12.0±0.2 14.0±0.3
3	Zagatala514 Control Salt	86±1 50±4	8.2±1.4 3.9±1.5	2.1±0.2 1.3±0.1	0.54 0.45	77 66	0.011 0.019	10.8±0.5 13.0±0.2
4	Zagatala420 Control Salt	90±2 60±4	9.8±1.6 4.7±1.3	2.4±0.2 1.6±0.1	0.58 0.55	99 84	0.011 0.014	7.0±0.1 8.8±0.3
5	Gurur Control Salt	90±1 85±2	11.4±1.3 7.3±1.5	2.8±0.3 2.4±0.1	0.75 0.72	78 74	0.021 0.018	12.2±0.2 13.2±0.1
6	Gurur x Zagatala68 Control Salt	95±2 89±1	8.5±1.8 5.7±1.7	2.1±0.1 1.9±0.1	0.80 0.75	83 80	0.025 0.023	12.6±0.4 18.0±0.5

* Each value represents the mean ± SD (standard deviation) for the mean n =3 independent experiments p = 0.05.

Table 4: Effect of NaCl on elements of the yield structure of durum and bread winter wheat varieties under the conditions of a growing experiment

wheat varieties	Plant height, (sm)		number of grains per ear		grain weight, (g)		Crop losses (%)
	control	NaCl	control	NaCl	control	NaCl	
Gobustan	90 ± 2	68 ±1	62±3	45±4	3,6	1,4	62
Mirbashir-128	105 ± 4	73 ±1	42±2	18±1	4,6	1,2	74
Barakatli-95	85 ± 2	75 ±2	68±3	29±2	3,5	1,7	52
Garabagh	80 ± 2	62 ±1	41±1	32±2	2,2	1,5	32
Gyrmyzygul	72 ± 1	57 ±2	46±2	22±1	3,1	2,4	23
Shark	110 ± 4	70 ±3	32±2	19±1	4,2	1,4	67
F_2 Hybryds							
Garabagh x Gobustan	120 ± 3	65 ±2	52±3	36±2	1,4	1,1	22
Gobustan x Barakatli-95	76 ± 2	100±4	32±2	20±1	2,8	2,2	22
Gobustan x Gyrmyzygul	118 ± 4	70 ±2	34±1	27±2	2,9	2,3	21
Barakatli-95 x Gobustan	78 ± 1	63 ±1	40±2	21±1	1,9	1,6	10
Gobustan x Qarabagh	80 ± 1	60 ±2	38±1	34±2	4,2	2,8	34
Garabagh x Mirbaşir-128	90± 2	60 ±1	54±3	33±3	1,9	1,7	11
Garabagh x Shark	90 ± 2	70 ±2	60±2	22±2	3,2	1,3	60

* Each value represents the mean ± SD (standard deviation) for the mean n =3 independent experiments p = 0.05.

Discussion

Salinity could affect chlorophyll concentration of leaves through inhibition of synthesis of chlorophyll or an acceleration of its degradation. Impairment of the carboxylation capacity, which in turn inhibits electron transport, is indicated by the measurements of chlorophyll fluorescence. A reduced quantum yield may result from a structural impact on PS II although some authors [Lu, C.M., *et al*., 2002] [9] found PS II to be highly resistant to salinity stress. Salinity has been concluded to affect reaction centers of PS II either directly or via an accelerated senescence. High external salt concentrations could affect thylakoid membranes by disrupting lipid bilayer or lipid-protein associations and thus, impair electron transport activity. The efficiency of the photochemical conversion of the PS II energy decreased with increasing salt concentrations. Some authors indicate the decrease of the root system function in plants exposed to salt stress. They assumed a more important role of toxic effects of ions [Wang Wen – Yuan, *et al*., 2012] [10].

Previous measurement of linear electron transport revealed that salinity does not affect electron transport in wheat. Effect of salinity on rate of electron transport could, however, be species specific [Lutts. S., et al, 1996] [11].

In general, the growth and development of plants depend on the process of photosynthesis in their green organs. Therefore, environmental stressors affecting photosynthesis also affect growth and development [Villora., *et al*., 1997] [12]. A positive correlation between the rate of photosynthesis and productivity has been found in various plants under salinity [Perez-Alfocea., *et al*., 1993].

The decrease in RWC due to stress indicates that the cell does not have the turgor necessary for the tension process to take place [Katerji., *et al*., 1997] [14]. The response of plants to stressors is different, depending on the netic material. Thus, the genetic material regulates the speed and consistency of protein synthesis required under stress. Some difficulties in the cultivation of salt-tolerant forms are attributed to the complexity and polygenic nature of genes. It known that under the salt stress, the external water potential decreases, the absorption of biogenic metal ions by the roots becomes difficult, and the chlorine and sodium ions have a toxic effect on plant metabolism. These three possible effects of salt stress have a detrimental effect on plant growth, development and yield. [Muhammad, *et al*., 2015, Munns, *et al*., 2005] [18, 19]. Osmotic stress is associated with the accumulation of ions in the soil solution, while malnutrition and the specific effects of ions are associated to the accumulation of ions, mainly sodium and chloride, to toxic levels which inhibits the availability of other important elements such as calcium and potassium [19]. Toxic levels of sodium in plant organs damage biological membranes and subcellular organelles, reducing growth and causing abnormal development before plant death. Several physiological processes, such as photosynthesis, respiration, starch metabolism and fixation of nitrogen also disrupted in salt conditions, which leads to a decrease in crop productivity. In response to this, the plant synthesizes low molecular weight solutes, including soluble carbohydrates for better absorption of water during salinity. Genotypes with a powerful genetic apparatus cope with this task and grow well in salt conditions. In the process of evolution, protective mechanisms against environmental stressors are formed in all organisms, including plants. Therefore, when assessing tolerance to stress factors, it is necessary to consider the individual characteristics of each plant genotype [Azizov, *et al*., 2019] [15]. NaCl (0.98 %) in the soil has been found to affect physiological parameters of parental and hybrid forms differently.

Conclusion

Negative effects of salt stress were observed in the content of photosynthetic pigments, photochemical activity of PS II and RWC in parental and hybrid forms. The content of chl a, chl b, the activity of PSII, RWC and grain yield were higher in the parental forms, Garabagh, Gobustan, Barakatli -95, and in the hybrids, Garabagh x Gobustan, Barakatli 95 x Gobustan, Gobustan x Gyrmyzygul-1 and Garabagh x Mirbashir-128. Due to these advantages, using these varieties for future research on the development of salt-tolerant forms can be considered expedient.

At concentrations of NaCl solutions of 150 and 200 mmol decreased seed germination, pigments content, and photosystem II activity in seedlings of maize genotypes. Varieties Gurur and Zagatala- 68 and hybrid Gurur x Zagatala -68 were resistant to 200 mmol salt concentration.

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