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THE EFFECT OF VARIOUS NaCl CONCENTRATIONS ON MORPHOPHYSIOLOGICAL PROPERTIES OF BREAD WHEAT SPROUTS (*TRITICUM AESTIVUM* L.)

Aim. The effects of various NaCl concentrations on germination energy, germination percentage of seeds and morpho-physiological indices of seedlings have been determined in bread wheat (*Triticum aestivum* L.) varieties. **Methods.** Wheat seeds were germinated at 0, 100, 150, 200 mM concentrations of NaCl using the roll method. **Results.** The delay of the growth of seedlings and root system was observed with increasing salt concentrations in all samples. The effect of various salt concentrations on separate varieties was different. **Conclusions.** The highest indices of the development of coleoptiles and root system and the highest amount of chlorophyll were detected at 150 and 200 mM concentrations of NaCl in the Mirbashir-128 variety. The Bezostaya and Nurlu-99 varieties manifested medium tolerance, while the Sheki-1 variety was relatively less tolerant.

Keywords: NaCl, wheat varieties, germination energy, germination percentage, chlorophyll.

Soil salinization is one of the main stress factors limiting plant growth, development and productivity. In addition to natural salinization, careless irrigation of agricultural plants also causes a secondary salinization [1]. Salinity is known to form high osmotic potential and limits the water absorption by seeds, and Na⁺ and Cl⁻ ions accumulated in developing seeds have a toxic effect. It leads to the violation of enzymatic processes of carbohydrate and protein synthesis in seeds. After the root system formation the rate of the water uptake by plant roots decreases and penetration of Na⁺ and Cl⁻ ions into plant cell weakens cell ultrastructure and chloroplast photosystem. The structure of the plasmatic membrane and the function of the ion pumps are disturbed under the influence of Na⁺ ions. In many cases this results in the imbalance between Na⁺ and Cl⁻ ions in cytosol [2–6].

Defense mechanisms against environmental stress factors were formed in living organisms during the evolution process and plant tolerance to salinity occurred due to various defense mechanisms.

Some soluble substances – osmolites are synthesized in cells in order to protect plants from stress. These mechanisms provide plant tolerance against salt ions and further realize repair of cell damage [7].

High salt concentrations decrease solubility of small amounts of food elements obstructing their access to plants and destructively affect normal physiological processes, increasing the formation of reactive oxygen species (ROS) [8]. Toxic effects of Na⁺ and Cl⁻ ions on intracellular mechanisms of regulation processes and their relation to pigments, especially in the view of the initial molecular damage caused by stressors of this type have not been studied sufficiently [9].

The main purpose of the presented research was to evaluate salt tolerance of bread wheat genotypes according to the morphometric indices in the germination stage and to establish changes in leaf water regime, the amount of photosynthetic pigments, and changes in PS II activities of chloroplasts.

Materials and methods

Highly productive bread wheat (*Triticum aestivum* L.) varieties (Mirbashir-128, Sheki-1, Bezostaya, Nurlu-99) were used as the objects of the study.

Seeds were germinated using the roll method for studying the effect of salt on morpho-physiological indices of wheat seedlings grown at various salt concentrations [5, 6]. Wheat genotypes were screened for salt tolerance using NaCl solutions of various concentrations. For this purpose seeds of each sample were germinated on the wet filter paper at 20–22^oC temperature, for 3 days in the dark, and for 11 days at the photoperiod of 12 hours light/12 hours dark. Germination ability of wheat embryo under salt stress, average length of roots and shoots were measured for 14 days and tolerance of the varieties were evaluated according to morphometric parameters. Germination ability – germination energy and germination percentage

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were determined after 3 and 7 days of germination, respectively, for each variant of wheat genotypes [10]. Salt effects on physiological parameters were studied in 14-day-old leaves.

Relative water content (RWC) of leaves was determined according to Tambussi et al. [11]. Water parameters were estimated according to the formula:

$$\text{RWC} = 100 \% (\text{FW} - \text{DW}) / (\text{TW} - \text{DW})$$

where:

FW – fresh weight

TW – turgor weight

DW – dry weight

Chlorophyll was extracted from leaves by 96 % ethanol, and chlorophyll a, chlorophyll b and carotenoids were measured spectrophotometrically at 665 nm, 649 nm and 440 nm, respectively. Amounts of pigments were determined according to the method of Wintermans [12] (I.F. Wintermans A. De. Mots, 1965).

The concentrations of pigments were estimated according to the absorption at 665 nm, 649nm and 440 nm using the following formulae:

$$C_a = 13.70 \cdot A_{665} - 5.76 \cdot A_{649};$$

$$C_b = 25.80 \cdot A_{649} - 7.6 \cdot A_{665};$$

$$C_{a+b} = 6.10 \cdot A_{665} + 20.04 \cdot A_{649} = 25.1 \cdot A_{654};$$

$$C_{car} = 4.695 \cdot A_{440,5} - 0.268 (C_{a+b});$$

where:

C_a , C_b , C_{car} – pigment concentration (mg/ml);

A_{665} , A_{649} , A_{440} – absorption

Fluorescent indices of leaves were measured using MINI-PAM (photosynthesis yield analyzer, Germany) device. Using the formulae $F_v = F_m - F_0$ and F_v / F_m the efficiency of photochemical conversions in PS II was determined (13).

F_0 – fluorescence of dark-adapted leaf;

F_m – fluorescence of light-saturated leaves;

$F_v = F_m - F_0$ – variable fluorescence;

F_v / F_m – the efficiency of photochemical conversions in PS II

Data analysis and statistical analysis was conducted in Microsoft Excel. Statistical analysis was performed with the aid of the Statgraphics Plus 5.1 statistical package. The means of values were compared by Duncan's multiple range test ($p = 0.05$).

Results and discussion

The study of the effect of various NaCl concentrations on the germination ability of wheat genotypes showed that stress caused a decrease in the

germination energy of embryos in 3-day-old wheat seedlings and a less decrease compared with other varieties was observed in the Mirbashir-128 variety.

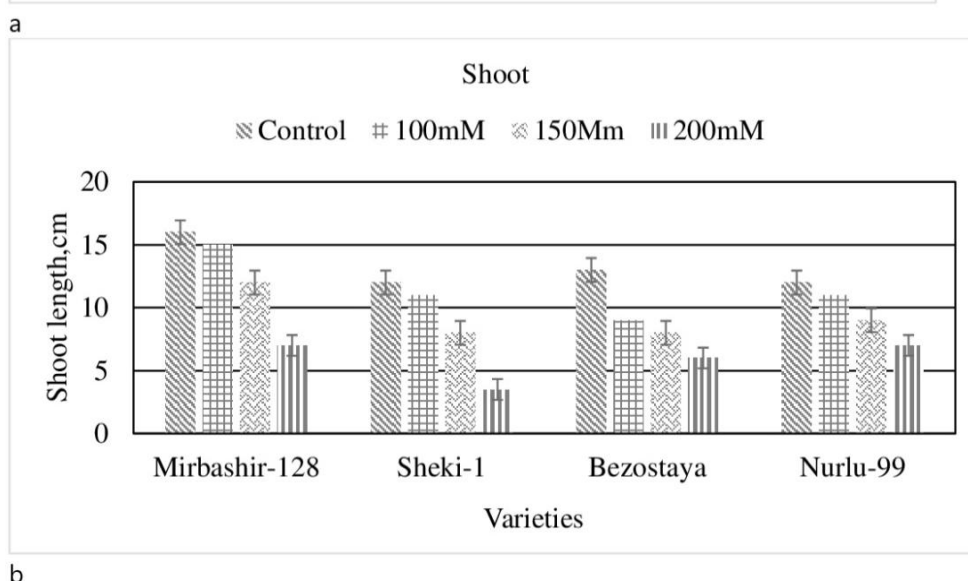
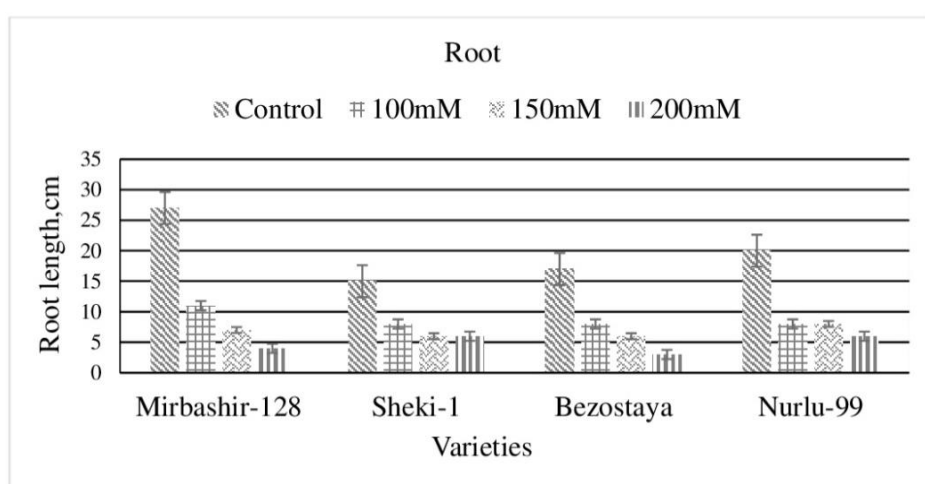
Thus, as seen in the table 1, under chloride salinity germination energy of bread wheat genotypes in control variants decreased from 93 % to 63 % and 100 mM, 150 mM and 200 mM concentrations of NaCl decreased the germination energy from 80 % to 60 %, from 60 % to 53 % and from 53 % to 27 %, respectively. But, all varieties in every variant have maximum germination (Table 1). Effects of various NaCl concentrations on the germination of bread wheat genotypes energy. Only in the Sheki-1 variety a relatively small decrease (27 %) in the germination energy was observed at 200 mM NaCl.

A general development of the root system and a close relation to some traits of plants indicate the importance of these parameters. The change in linear parameters of the growth processes expresses salt tolerance of varieties more precisely, compared with germination indices of seeds, which is related to the general tolerance of the plant. The delay in plant growth under unfavorable conditions, which occurs due to suppression of cell pressure and tension, can be considered as the defense reaction of the plant. Tolerant forms adapt faster to stress and recover their development (6). The effect of various salt concentrations on the growth of shoots and roots shows that first the root system and then above-ground organs of the plant are damaged. According to some authors it occurs due to the different tolerance of separate plant organs (14).

Fig. 1 (a, b) shows dependence between bread wheat genotype indices (length of roots and shoots) and salt concentration for 14 days. The growth dynamics of roots and shoots during 14 days showed that dynamics of the development of all wheat genotypes was normal in the germination phase and the germination rate of shoots decreased with increasing salt concentration compared with control. The growth of shoots and roots of all samples delayed with increasing salt concentration. At the same time the influence of various salt concentrations on separate varieties was different. Thus, at 150 mmol and 200 mmol concentrations of NaCl the highest growth indices of coleoptiles and root system was observed in the Mirbashir-128 variety. Moderate tolerance was detected in Nurlu-99 and Sheki-1 showed relatively weak tolerance.

Table 1. Effects of various NaCl concentrations on the germination of bread wheat genotypes

Germination ability of bread wheat varieties depending on salt concentration								
Varieties	NaCl, mM				NaCl, mM			
	Control	100	150	200	Control	100	150	200
	Germination energy, %				Germination percentage, %			
Mirbashir-128	93	80	60	53	100	100	93	80
Sheki-1	67	60	53	27	100	93	87	60
Bezostaya	73	73	60	27	100	87	87	67
Nurlu-99	87	80	60	53	100	100	73	67



b

Fig. 1. (a, b). Indices (the length of roots and shoots, for 14 days) of bread wheat genotypes grown at various salt concentrations. Each bar represents mean \pm sd (standart deviation) for average of n=3 independent experiments; P=0.05.

RWC was studied in 14-day-old shoots of bread wheat genotypes grown at various salt concentrations (fig. 2). As seen in the figure in leaves of 14 day-old shoots RWC significantly decreased with increasing salt concentrations. But there was no pronounced difference between

dynamics of changes in RWC depending on salt concentrations in the studied wheat genotypes.

However, RWC values for the Mirbashir-128 variety were higher (by 18 %) compared with Sheki-1 under high salt concentration. The obtained

results show that Mirbashir-128 is more tolerant to salt compared with other varieties.

Changes in plant leaf pigments provide an important information on physiological status and adaptation of the plant to the changing environment [15]. The purpose of our research was the evaluation of the effect of various NaCl concentrations on photosynthetic pigments in wheat leaves as the indicator of the degree of stress factor influence on the plant growth. An increase in salt concentration causes the disturbance in ion homeostasis in

plants at the cell and whole plant level. In its turn it leads to various toxic effects manifested as cytoplasmic biopolymer damages. The effect of salt on the amount of green pigments in 14-day-old leaves showed that the changes in photosynthetic pigments are correlated with a decrease in RWC. Total amount of chlorophyll decreased in all varieties with increasing salt concentrations. But this parameter was higher for the Mirbashir-128 genotype compared with others (Table 2).

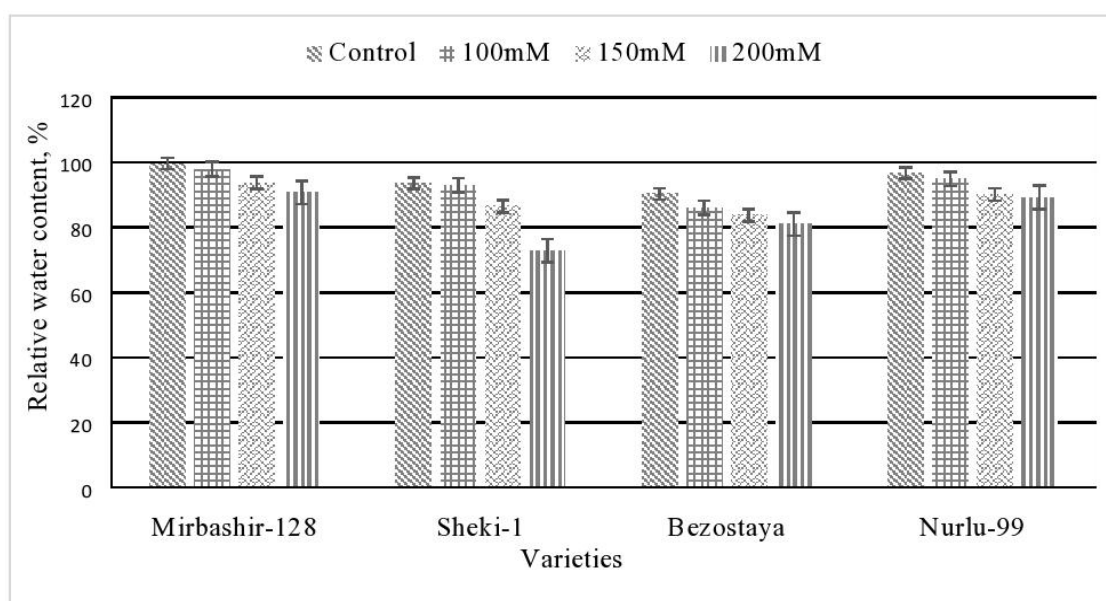


Fig. 2. RWC in 14-day-old leaves of bread wheat varieties grown under various NaCl concentrations. Each bar represents mean \pm sd (standard deviation) for average of n=3 independent experiments; P=0.05.

Table 2. The influence of various NaCl concentrations on the amounts of chlorophyll and carotenoids (mg/g), and the energy conversion efficiency of PS II (Y).

No	Varieties	NaCl, mM	C _a	C _b	C _{a/b}	C _{kar}	Y
1	Mirbashir-128	0	16.0	4.8	3.3	3.3	0.79
		100	10.9	3.9	2.8	3.2	0.74
		150	9.4	3.8	2.5	2.1	0.79
		200	5.0	2.0	2.5	1.2	0.6
2	Sheki-1	0	9.5	3.7	2.5	2.6	0.78
		100	7.2	2.3	3.1	1.7	0.79
		150	6.3	2.2	2.8	1.6	0.71
		200	4.7	1.7	2.7	1.5	0.70
3	Bezostaya	0	11.0	4.3	2.5	2.9	0.78
		100	11.0	4.3	2.5	2.9	0.79
		150	5.4	1.7	3.2	1.3	0.8
		200	1.7	0.7	2.4	0.5	0.7
4	Nurlu-99	0	8.9	3.2	2.8	2.0	0.81
		100	7.6	2.6	2.9	1.6	0.79
		150	7.1	2.5	2.8	1.7	0.79
		200	7.0	2.2	3.1	1.6	0.77

As seen in the Table 2, an increase occurred in the chlorophyll a/b ratio and carotenoid amounts under the NaCl influence. According to some authors, amounts of chlorophyll b decreased more markedly in leaves of various wheat genotypes exposed to salt and drought stress [16]. Such a pattern we observed only in Nurlu-99. 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 [17]. Our results confirmed that salt stress effects

are initially expressed themselves in changes occurring in seeds.

Conclusions

Thus, the research performed showed that salt stress negatively affects germination ability, RWC of leaves, amounts of photosynthetic pigments and the PS II activity of wheat genotypes. The obtained results confirmed that the tolerance to extreme stress conditions is a consequence of complex physiological adaptive responses of plants.

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ВПЛИВ РІЗНИХ КОНЦЕНТРАЦІЙ NaCl НА МОРФОФІЗІОЛОГІЧНІ ВЛАСТИВОСТІ ПРОРОСТКІВ М'ЯКОЇ ПШЕНИЦІ (*TRITICUM AESTIVUM* L.)

Мета. Досліджено вплив різних концентрацій NaCl на енергію проростання, відсоток проростання насіння і морфофізіологічні показники проростків у генотипів м'якої пшениці (*Triticum aestivum* L.). **Методи.** Насіння пшениці пророщували за 0, 100, 150, 200 мМ концентрацій NaCl з використанням рулонного методу.

Результати. Зі збільшенням концентрації хлористого натрію спостерігалася затримка росту проростків і кореневої системи у всіх зразках. Різні концентрації солі на окремі сорти впливали по-різному. **Висновки.** Вищі показники розвитку проростків і кореневої системи і найбільшу кількість хлорофілу було виявлено при 150 і 200 мМ концентраціях NaCl у сорту Мірбашір-128. Сорти Безостая і Нурлі-99 проявляли середню толерантність, у той час як сорт Шекі-1 був менш толерантним.

Ключові слова: NaCl, сорти пшениці, енергія проростання, відсоток проростання, хлорофіл.

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ВЛИЯНИЕ РАЗЛИЧНЫХ КОНЦЕНТРАЦИЙ NaCl НА МОРФОФИЗИОЛОГИЧЕСКИЕ СВОЙСТВА ПРОРОСТКОВ МЯГКОЙ ПШЕНИЦЫ (*TRITICUM AESTIVUM* L.)

Цель. Исследовано влияние различных концентраций NaCl на энергию прорастания, процент прорастания семян и морфофизиологические показатели проростков у генотипов мягкой пшеницы (*Triticum aestivum* L.).

Методы. Семена пшеницы проращивали при 0, 100, 150, 200 мМ концентрациях NaCl с использованием рулонного метода. **Результаты.** С увеличением концентрации хлористого натрия наблюдалась задержка роста проростков и корневой системы во всех образцах. Различные концентраций соли на отдельные сорта влияли по-разному. **Выводы.** Более высокие показатели развития проростков и корневой системы и наибольшее количество хлорофилла было обнаружено при 150 и 200 мМ концентрациях NaCl у сорта Мирбашир-128. Сорта Безостая и Нурлу-99 проявляли среднюю толерантность, в то время как сорт Шеки-1 был относительно менее толерантным.

Ключевые слова: NaCl, сорта пшеницы, энергия прорастания, процент прорастания, хлорофилл.