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ORIGINAL ARTICLE

Effect of osmotic stress on the development of durum wheat seedlings

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We performed a laboratory experiment to compare the response of the two genotypes of spring durum wheat to excessive chloride salinity and osmotic stress. The cultivars differed in field tolerance to drought when grown under conditions of the steppe zone of the Altai Territory, Russia. To simulate osmotic stress, solutions of polyethylene glycol 6000 and sodium chloride were used, whose osmotic potential was 11 MPa. The control was distilled water. We evaluated the following traits: seed germination, shoot length, root length, the number of primary roots, root fresh weight, shoot fresh weight. The studied genotypes turned out to be more sensitive to stress triggered by a high content of sodium chloride in the environment. The main damaging factors under the action of excess salts are complex osmotic and toxic effects. A higher seed germination and a more developed root system of seedlings of 'Bezenchukskaya 210', as compared to '12S1-14', confirm the ability of the drought-tolerant genotype to osmotic adjustment under osmotic stress.
Key words: durum wheat, osmotic stress, drought, salinity, seed germination, seedling, shoot and primary root traits, sodium chloride, polyethylene glycol 6000

Drought and soil salinity are the main abiotic factors limiting crop production, reducing yields below their genetic potential (Jones, 2007; Ahmad et al., 2014; Al-Yassin & Khademian, 2015). Increasing plant productivity to the genetically possible under stress is a challenge for breeders (Khayatnezhad et al., 2010). High concentrations of salts in the soil solution worsen cell metabolism and photosynthesis; impose osmotic stress on cellular water relations, increasing the toxicity of sodium in the cytosol (Sayar et al., 2010). Seed germination depends on the external osmotic potential under stress. The osmotic potential prevents the absorption of water due to the toxic effects of Na^+ and Cl^- ions both at the time of seed germination and during the further development of the seedling (Murillo-Amador et al., 2002). The accumulation of soluble salts in the soil leads to an increase in the osmotic pressure of the soil solution, which can limit the absorption of water by seeds or plant roots. The damage caused by high salt concentrations is due to reduced water availability, toxicity or specificity of ions, as well as imbalance in plant nutrition caused by such ions (James et al., 2006).
Polyethylene glycol (PEG), which is widely used for modeling osmotic stress, is a non-ionic water polymer that practically does not penetrate into plant cells (Kawasaki et al., 1983). On the contrary, Na^+ and Cl^- ions penetrate into plant cells and can accumulate in vacuoles of tolerant plants or cytoplasm of unstable genotypes (Genc et al., 2007). Seed germination and various parameters of seedling development are the common criteria for the breeding salt-tolerant and drought-tolerant cultivars growing under conditions of salinity and moisture deficit (Khan et al., 2013; Mordi & Zavareh, 2013). The decrease in these indicators, as a rule, leads to significant losses in crop yields. The purpose of the study is evaluating the response of the genotypes of spring durum wheat to drought and salinity modeling in a laboratory experiment.

Methods

The experiment was performed in 2017 in the Altai Center for Applied Biotechnology (ACAB) at the Altai State University, Russia. Two samples of *Triticum durum* Desf. served as the material for the study, the seeds of which were kindly provided by the staff of the Lab of Durum Wheat Breeding, the Altai Research Institute of Agriculture, Russia. Genotypes differed in drought tolerance when grown in the field of forest-steppe zone of the Altai Territory. The cultivar 'Bezenchukskaya 210' is resistant to lack of moisture, and the line '12S1-14' is susceptible to water scarcity. Seeds germinated by the method of paper rolls, pre-treating 1% $KMgO_4$ solution. PEG 6000 and sodium chloride solutions, whose osmotic potential was 11 MPa, were used to simulate osmotic stress. The control was distilled water. The paper rolls with seeds were moistened with equal amounts of desired osmotic solutions or distilled water and incubated in the dark for 5 days at a temperature of 25 ± 1 °C with an average relative humidity of 80 ± 1 % throughout the experiment. The following traits were evaluated: seed germination, shoot length (SL), root length (RL), the number of primary roots, root fresh weight (RFW), shoot fresh weight

(SFW). The experiment was conducted in five replicates of 100 seeds per replication of each treatment. The results were statistically processed using the Microsoft Office Excel 2010 application software.

Results and Discussion

Comparison of growth parameters of seedlings of durum wheat genotypes contrasting in field drought tolerance showed that the samples differed significantly in response to high sodium chloride content and osmotic stress caused by polyethylene glycol. It confirms the possibility of studying the mechanisms of tolerance to salinity and drought due to genotypic variability of samples (Gregorio et al., 2002).

The ability of seeds to germinate is a critical moment to obtain seedlings with growth energy. Seeds are more sensitive to stress than mature plants, including due to the impact of dynamic environment near the soil surface (Dodd & Donovan, 1999). Figure 1A shows seed germination in the absence of stress (control), as well as the effect of various treatments. In control conditions, the germination percentage accounted for 81.4 ± 6.7 and 93.7 ± 4.2 for genotypes '12S1-14' and 'Bezenchukskaya 210', respectively. The increase in osmotic pressure in the solution led to a significant decrease in the germination of both genotypes. The effect of sodium chloride was slightly less inhibitory than the effect of PEG 6000. The stress caused by the high content of NaCl in the medium led to a decrease in the trait to 31.7 ± 9.3 % ('12S1-14') and 54.1 ± 9,1 % ('Bezenchukskaya 210'). Seed germination under conditions of moisture deficiency caused by polyethylene glycol was 23.3 ± 4.4 % and 31.7 ± 4.8 % in '12S1-14' and 'Bezenchukskaya 210', respectively.

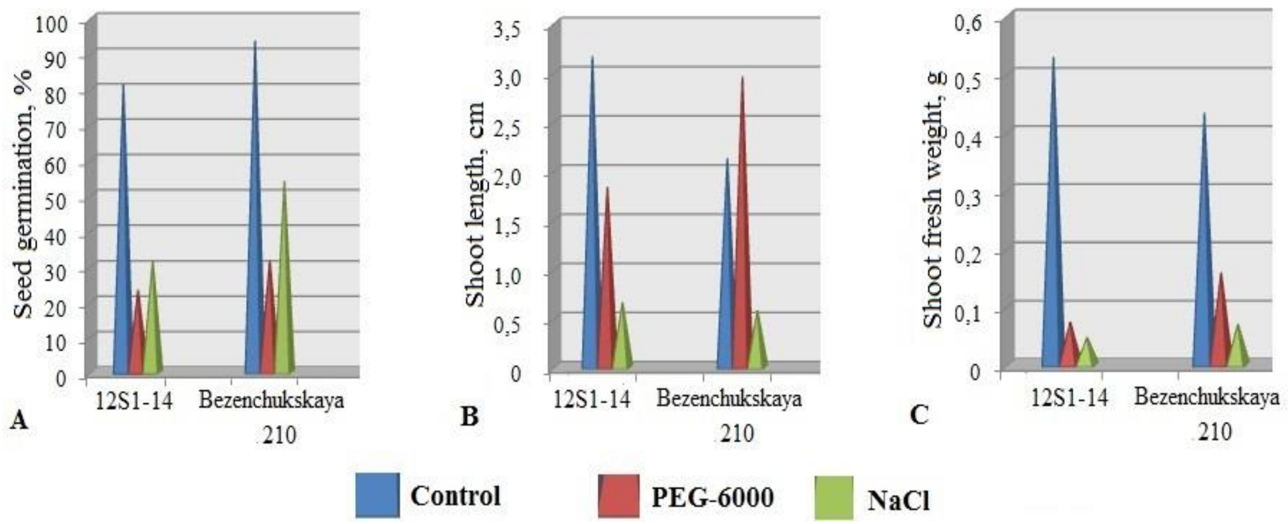

Figure 1. Seed germination, shoot length and shoot fresh weight of spring durum wheat under conditions of salinity and osmotic stress

The further development of seedlings of contrasting genotypes occurred in different ways depending on the stressor. So, the average length of the shoot of the drought susceptible sample '12S1-14' (grown with the addition of PEG 6000) decreased by more than 1.5 times compared with the control, reaching 1.84 ± 0.80 cm. When exposed to sodium chloride, the trait decreased by 4.8 times relative to the control, amounting to 0.66 ± 0.22 cm (Fig. 1B). The drought-tolerant cultivar 'Bezenchukskaya 210', on the contrary, showed a significant increase in the shoot length on medium containing PEG 6000. The effect of sodium chloride, as for the previous sample, led to inhibition of the trait. The shoot fresh weight under stress significantly decreased relative to the control, regardless of the genotype and treatment, with statistically significant differences in the influence of different osmotic substances were not observed (Fig. 1C).

The development of the root system in contrasting genotypes under the influence of polyethylene glycol and sodium chloride differed significantly (Fig. 2A, 2B, and 2C). In the absence of stress the line '12S1-14' formed maximum root length (5.89 ± 0.63 cm) with a smaller number (2.92 ± 0.08 pcs.). In the cultivar 'Bezenchukskaya 210' the development of the root system, on the contrary, was due to the formation of a larger number of primary roots (3.50 ± 0.17 pcs.), yielding in length to the previous sample. The action of polyethylene glycol led to a significant decrease in all parameters in the genotype '12S1-14'. So the root length, their number and fresh weight were decreased by 59.93 %, 82.88 % and 16.40 %, relative to the control, respectively. Higher values of RFW and number of roots in comparison with the control in 'Bezenchukskaya 210' indicate the ability of drought-tolerant sample to withstand stress caused by PEG-6000.

Sodium chloride showed an inhibitory effect on the development of the root system of both samples, reducing the average length of the roots by almost half. The root fresh weight was decreased by 60 % and 76 % in both drought-tolerant and susceptible genotype. The least variable trait was the number of roots, not differing much depending on the type of treatment in the line '12S1-14' and slightly yielding to the control and the variant with PEG-6000 in 'Bezenchukskaya 210'.

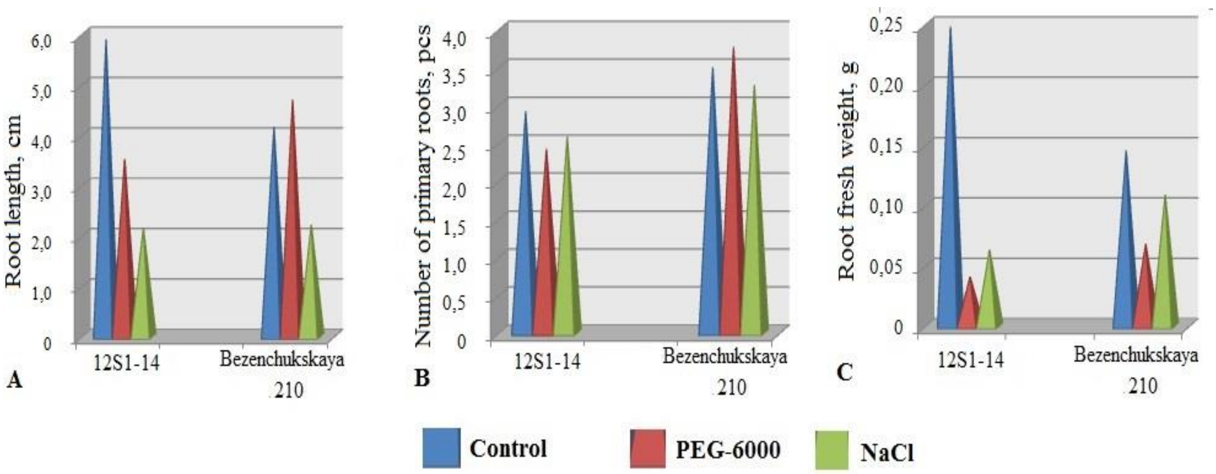

Figure 2. Root length, number of primary roots and root fresh weight of durum wheat seedlings under conditions of salinity and osmotic stress

Thus, the behavior of durum wheat genotypes under conditions of osmotic stress, modeled by a high molecular weight substance of non-ionic nature (PEG 6000), conforms to their ability to withstand the moisture deficit in the field. The drought-tolerant cultivar 'Bezenchukskaya 210' showed a significantly lower decrease relative to the control of various traits of shoots in comparison with the line '12S1-14'. The inhibitory effect of this osmotic on the root system was less pronounced. According to the literature data, in the conditions of water deficiency at the same value of water potential, leaf growth is limited to a greater extent than the growth of roots, due to the greater ability of the latter to osmotic adjustment (OA) (Ober & Sharp, 2007). Biochemical analysis of apical areas of primary roots of maize seedlings exposed to water stress revealed a significant increase in the amount of proline, which plays a significant role in osmoregulation (Sharp et al., 2004). In addition, the decrease in leaf growth activity, preceding the slowdown of photosynthetic processes in drought conditions, causes the outflow of the carbohydrate excess to the roots, also supporting osmotic adjustment and root growth (Blum, 2011). The impact of excessive salinization on various traits of seedlings of the studied samples was different. There was a decrease in the level of all considered features regardless of the level of field drought resistance of genotypes.

It is known that drought and salinity are physiologically related, because both induce osmotic stress. Most of the metabolic reactions leading to negative consequences are to some extent similar with each other (Djibril et al., 2005). For example, common to these stressors are a violation of water metabolism of plants and inhibition of growth by stretching of divided cells (Veselov, 2009). However, it should be noted that the effects of salt stress include both saline-specific and osmotic components. The toxic component is associated with the accumulation of ions in the cytoplasm, and water deficiency is due to the presence of excess ions in the soil (Munns et al., 2006; Sayar et al., 2010). The main attention of researchers is drawn to the study of mechanisms that provide ion homeostasis during salinization. In this case, the osmotic component is perceived rather as a factor masking the manifestation of the toxic component (Munns et al., 2006). However, these components are bit interconnected: ions enter the plant with a transpiration stream, and their accumulation disrupts water exchange (for example, the functioning of the stomatal apparatus) (Veselov et al., 2008).

The results of our studies confirm various mechanisms of tolerance to osmotic stress caused by moisture deficiency and high salt content in the medium. One of the factors of tolerance to the lack of water is the ability of the genotype to osmotic regulation, which allows to continue growth under stress. In this case, the osmotic adjustment is achieved by the synthesis and accumulation of organic compatible osmolites in the cytoplasm, which causes a decrease in the osmotic potential of the cytosol. Typical compatible substances are sugar, amino acids and their derivatives (proline and/or glycinebetaine), alcohols (mannitol) and other low molecular weight metabolites (Blum, 2011; 2017). It is known that inorganic ions can also play a role in osmotic adjustment, as it was shown for wheat, where one of the components of OA were potassium ions (Morgan, 1992). Under salt stress, the process of osmotic adaptation is carried out by absorption and accumulation of inorganic ions, mainly Na^+ and Cl^- (Alian et al., 2000). However, the high concentration of sodium ions as a result of osmotic regulation is toxic to the cell, which has adversely affects for the growth and development of the plant (Blum, 2011).

Salinization can also affect seed germination, facilitating the penetration of toxic ions, which can change their enzymatic or hormonal status (Smith & Comb, 1991). According to some researchers, the inhibition of seed germination is associated with both osmotic and toxic effects of excess salts (El-Hendawy et al., 2005). Since seed germination is more sensitive to salinization and drought than plant growth and development, greater tolerance of the crop, such as spring durum wheat, during germination can be considered as an adaptive feature of this species for saline or arid environments (Freeman, 1973). However, there is another point of view in the literature. J.D. Rhoades (1990) believes that plants may be relatively tolerant during germination, but later become more sensitive to the negative factors, confirming the opinion of J. Levitt (1980). J. Levitt stated the germination test was not always a good indicator for differentiating cultivars according to drought or salt tolerance. In our experiment, there was a significant decrease in seed germination of the studied samples regardless of the level of field resistance to drought. The inhibitory effect of PEG 6000 was more pronounced, resulting in more than three-fold differences with the control values. However, the negative impact of stress factors of different nature on the genotype with high field drought resistance was much weaker.

Conclusions

The genotypes of spring durum wheat differing in drought tolerance under field conditions, turned out to be more sensitive to stress triggered by a high content of sodium chloride in the environment. The main damaging factors under the action of excess salts are complex osmotic and toxic effects. A higher seed germination and a more developed root system of seedlings of 'Bezenchukskaya 210', as compared to '12S1-14', confirm the ability of the drought-tolerant genotype to osmotic adjustment under osmotic stress.

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