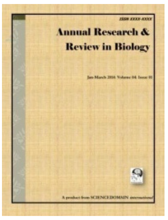

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Osmotic Stress Effect on Different Cytological Characters of Roots and Growth Parameters in Different Wheat Species

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Author's contributions

This work was carried out in collaboration between both authors. Author NVT designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author NAK wrote the protocol, managed the cytological analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To show how and why seedling growth parameters and different cytological characters of the roots of different wheat species may vary during osmotic stress conditions. To identify species that are tolerant to a lack of moisture in the early stages of ontogenesis.

Study Design: The experiments were conducted in the greenhouse of the Institute of Plant Biology and Biotechnology at 26℃±2℃ and at 3000 lux illumination. All experiments were performed in three biological replicates. At least 25 plants were studied in replication.

Place and Duration of Study: This study was conducted in the Laboratory of Cell engineering, Institute of Plant Biology and Biotechnology Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan in 2014.

Methodology: Plantlets of different species of wheat were grown for 7 days in culture water; then,

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for 72 hours, they were exposed to stress (a 17.6% sucrose solution). Control plantlets were grown in water. Growth, biomass accumulation, and parameters of root security were measured. Cytological examination of the root cells was carried out with squash preparation.

Results: The differences between the types of growth parameters and cytological characteristics of the root cells under stress are shown. The species identified that are tolerant to a lack of moisture in the early stages of ontogenesis are *T. dicoccum* Schuebl. and *T. aethiopicum* Jakubz.

Conclusion: As a result of the experiments shows a clearly expressed common nonspecific cytological responses of cells of the root system of cereals to osmotic stress - reduction of linear growth parameters, plasmolysis. At the same time are marked a variety of specific changes, which depend on the genotype of the test forms. Nonspecific response processes of cells in the root systems studied in various species of wheat to osmotic stress can offer recommendations as to which methods can be used to assess drought resistance among any species or varieties of wheat in the early stages of ontogenesis. The degree of species specificity studied in response to osmotic stress renders it possible to identify forms that are tolerant to a lack of moisture in the early stages of ontogenesis. Wheat species such as *T. dicoccum* Schuebl. and *T. aethiopicum* Jakubz, have the most stable indicators of the development of the root system of seedlings under osmotic stress; they can serve as valuable sources of these parameters when crossing them with cultivated varieties of wheat to improve drought resistance in modern varieties.

Keywords: Wheat species; drought tolerance; plantlets; growth; biomass; root development; cytological reactions.

1. INTRODUCTION

With the Earth's water resources under strain, its booming population growth, and its increasing desertification, the need to wring more crops out of dry land is becoming increasingly important [1,2]. Improved agricultural production systems are required to ensure high yield via the efficient and sustainable use of available natural resources. This prospect has called for a "blue revolution" [3,4] based on the core idea that we need to obtain "more crop per drop" [5].

How efficiently a plant draws water from the soil, how well cells retain water, how much water is released through leaf openings (called stomata), and the timing of flowering relative to the seasonal onset of drought all factor into drought tolerance [1]. Stress responses in plants are tightly coordinated with developmental processes, but the interaction of these pathways is poorly understood [6]. The effect of stresses on the plant appears as integrated processes, external signals, and internal responses of the plant organism. It is this dependence that manifests into drought resistance from both internal and external factors makes this issue difficult research [7]. Physiological, biochemical, and genetic experiments that have been conducted to study resistance to abiotic stresses led to a lack of understanding of how stress may be considered as a measurable phenotypic trait. Understanding the response of plants to water deficiency is crucial for predicting the effects of climate change on the productivity of major crops, as well as on ecosystem function [8,9].

Plant growth is an integral characteristic that reflects the degree to which plants adapt to their environmental conditions. Cell growth by stretching depends, first of all, on the osmotic potential of the plant, on the turgor and tensile properties of a cell-like wall, and on the availability of water in relation to the plant's body height. Cell growth stretching depends primarily on osmotic potential and turgor, cell wall extensibility, and the availability of water for growth [10,11]. The cessation of growth indicates the disappearance of the water potential gradient between the growing cells and xylem, but the compression of leaves indicates a reversal of the gradient; sustentacular cells then reverse the water flow, which has lower xylem water potential when compared to growing cells [12]. Therefore, the ability of plants in the early stages of their development to efficiently use moisture in low-water conditions – one of the most important biological and agronomic traits – as well as to engage in seedling growth reactions to stressful conditions, is one of the more visual indicators of change in their metabolism [13].

A common effect of abiotic stresses is to cause tissue dehydration. Such dehydration is caused by the imbalance between root water uptake and leaf transpiration. Under some specific stress conditions, the regulation of root water uptake is more crucial to overcome stress injury than the regulation of leaf transpiration [14]. Among the ways in which the absorption of water is improved by the plant are important mechanisms, such as an efficient root system, a high root-to-shoot ratio (R/S), a difference in the osmotic potential of the plants, and the conservation of water [15]. Root traits, such as rooting depth and root biomass, have been identified as the most promising plant traits in wheat for terminal drought tolerance [16]. The tolerant types generally performed better than other cultivars under drought conditions, mainly through maintaining higher water use efficiency, root viability, root elongation, or root production [17]. However, cytogenetic studies the root system of wheat plants under osmotic stress anywhere researchers almost never engaged. Such studies in the field are impossible to conduct and do not make sense, since sucking thin roots with an insufficient water supply causes them to lose turgor and almost immediately die. It has been difficult to unearth how roots cope with arid conditions. Unlike leaves, their workings are hard to observe [1]. In the laboratory, it was revealed that during drought and salinity conditions in the roots, the outer cells suffer; these include root cap cells that are in direct contact with the salt solution [18]. R Manns [18] noted that the level of stress (175mM of NaCl equivalent and above) can cause plasmolysis in the epidermal cells of roots. The processes that occur within the cells of *S. altissima* during epidermis plasmolysis and root bark halophyte depress the plant's growth in a specific NaCl concentration in the nutrient solution, as was observed by Tsydendambaev et al. [19]. Such laboratory experiments are certainly interesting and relevant to providing a better understanding of the mechanisms of the primary root system's response to stress.

The tribe *Triticeae* Dum., which belongs to the most important food crop (soft wheat) (*Triticum aestivum* L.), undergoes huge potential stress [9]. Wild representatives of tribes, some of which are even halophytes that grow in a wide range of conditions around the world, have great genetic variation that causes considerable variability in their resistance to stress [20]. Drought tolerance among wild species of wheat is almost never used to increase plant resistance to culture-related forms of stress, as resistance mechanisms inherent to wild relatives have hardly been studied. It is obvious that the biological responses of living organisms to the actions of stress-inducing factors have been evolutionarily formed during the long-term adaptation of plants to the environment, and they share a universal character. Tolerance to water deficits was evolutionarily relevant to the conquest of land by primitive plants [21]. Cytophysiological research on plants and animals has shown that in some cases, there is a correspondence between the body's cells that are resistant to this environmental factor and the "intensity" of this factor in the habitat type [22,23]. It is thus natural to assume that such cell resistance to the organism holds direct adaptive significance. Furthermore, the adaptive resistance of cells and tissues in some cases can lead to the development of a characteristic species. Understanding how wheat species manage water stress is important for the reclamation of drought-prone soils and crop production, and also for possibly discovering water stress-resistant genes to further develop drought resistance in this crop [24].

This article presents the results of experiments showing how and why certain parameters can change their seedling growth processes in the early stages of their ontogenesis under osmotic stress in wheat species with different resistance to drought. The important role of primary root systems in the early stages of ontogenesis for the normal growth and development of wheat plants under osmotic stress is shown.

2. MATERIALS AND METHODS

With respect to the materials in the study, the following wheat species were used: *T. monococum* L. (A^uA^u); *T. dicoccum* Shuebl. (A^uA^uBB); *T. polonicum* L. (A^uA^uBB); *T. aethiopicum* Jakubz. (A^uA^uBB); *T. compactum* Host. (A^uA^uBBDD); and *T. aestivum* L. (A^uA^uBBDD) – a sort Saratovskaya-29. The choice of these species for the experiment is due to the differences in their ploidy level, their genomic composition, and their degree of drought and salt tolerance, which was in accordance with various indicators that were studied previously [25-27]. However, before conducting this work, we considered the physiological condition of the plants of these species during abiotic stresses for such indicators as, for example, the development of a generative sphere and the parameters of water regime flag leaf and growing in salt conditions. In this paper, we consider the impact of the lack of moisture in the early stages of ontogenesis, studying the growth and development of the root system of seedlings among these species.

The experiments were conducted in the laboratory of the Institute of Plant Biology and Biotechnology; plants were kept in 26℃ and under illumination of 3000 lux. During a laboratory assessment of 10-day sprouts, the methods outlined by Udovenko [28] and Terletskaya et al. [29] were taken as the baseline. Plantlets were grown in 7 days of culture water, then for 72 hours, they were exposed to stress. Stressful conditions were created, exhibiting sprouts during water culture in a 17.6% sucrose solution (a firm AppliChem, Germany reactant). The concentration visually differentiates exemplars with respect to the growth and accumulation of biomass. Control plantlets were used, which were grown in water. Root security was determined as the ratio of the oven-dry weight of the roots to the dry weight of the shoot parts. All experiments were performed in three biological replicates. No fewer than 25 plantlets in each replication were studied.

A cytological examination was carried out using a squash preparation under the method described by Pausheva [30]. The material was fixed in the morning hours in freshly prepared Clark's reagent (three parts 96% ethyl alcohol: one part glacial acetic acid) and stored for 12–24 hours. All cytological examinations were studied via microscope (Micros; Austria), photographed with a video camera (YONGXIN OPTICS CAM V200) and analyzed with a computer program (YONGXIN OPTICS Scope Photo version 2.4) with an increase in lens ×40.

Statistical analysis of the data was performed using the method by Udolskaya [31]. The plus/minus values in the table indicate the relative error of the mean. The differences between treatment means were identified by t-test. The characters * and ** show the accuracy at 0.05 and 0.01 levels of significance, respectively.

3. RESULTS AND DISCUSSION

Research by Tyagi et al. [32] showed that the length of the root plants under osmotic stress is the most informative indicator of drought resistance of the seedlings, further prompting us to consider the length of the shoot and the ratio of the root/shoot linear parameters. Table 1 shows the results of an experiment that identified the negative effects of osmotic stress on the growth characteristics of the plantlets of various species of wheat

The experiment revealed significant species-specific differences in terms of the reduction of growth of the first leaf and the plant's roots under osmotic stress (leaf growth, from 56.0% to control in *T. aestivum* L. to 87.8% to control in *T. polonicum* L.; and roots, from 78.3% to control in *T. aestivum* L. to 157.9% to control in *T. compactum* Host.).

The smallest decline in leaf growth parameters differed between *T. polonicum* L., *T. aethiopicum* Jakubz., and *T. dicoccum* Schuebl. The smallest decrease in the growth parameters of primary roots occurred for *T. dicoccum* Schuebl. and *T. aethiopicum* Jakubz. For species such as *T. polonicum* L. and *T. compactum* Host., osmotic stress induced a significant increase in the linear growth of the primary roots (127.2% and 157.9%, respectively).

According to the data in Table 1, the percentage ratio of the linear dimensions of a root to a shoot in relation in the conditions of osmotic stress for different species of wheat also changes towards an increase. An increase in this ratio in such forms as *T. polonicum* L., *T. aestivum* L., and *T. compactum* Host. is the most frequently expressed. For forms such as *T. dicoccum* Schuebl. and *T. aethiopicum* Jakubz., it was found that in stressful conditions, a root/shoot ratio remains almost invariable.

The ability of wheat plantlets to accumulate biomass in stressful conditions is shown in Table 2.

According to the data in Table 2, the action of osmotic stress was reflected differently in terms of biomass accumulation, for both the shoots and roots of plantlets of different species of wheat. The greatest biomass accumulation of shoots in stressful conditions (in terms of the percentage related to control) was evident in *T. compactum* Host (100%), *T. aethiopicum* Jakubz. (90.3%), and *T. dicoccum* Schuebl (90%) forms, as well as in the biomass of germinal roots, as noted in *T. compactum* Host. (120%), T. dicoccum Schuebl. (111.1%), and *T. polonicum* L. (from 109.1%).

The tendency for the water content of the shoot and root system to decrease in stressful conditions was confirmed by the results of determination of water abundance. According to the data presented in Table 2, the greatest water content in relation to the control in shoots under

Table 1. Growth processes in various species of wheat plantlets in osmotic stress conditions (sucrose, 17.6%, 72 hours)

Species	Length, % of control		Ratio of root/shoot,%	
	Shoot	Root	Control	Stress
T. monococcum L.	80.9±4.5**	89.5±2.3**	56.3±3.0	62.3 ±3.1
T. dicoccum Schuebl.	82.8±6.4	93.6±2.5	41.6±2.4	47.0±2.3
T. polonicum L.	87.8±4.0	127.2±3.0**	43.7±1.9	63.3±3.2**
T. aethiopicum Jakubz.	85.5±4.2	92.6±2.5	34.3±2.0	31.7±2.0
T. compactum Host.	76.7±2.7**	157.9±3.8**	35.9±1.9	73.9±3.9**
T. aestivum L.	56.0±2.2**	78.3±2.0**	38.9±2.0	54.5±3.5**

*The characters * and ** indicate the accuracy of the t-test at 0.05 and 0.01 levels of significance, respectively*

Table 2. The relative increase of biomass and water content of different species of wheat plantlets under conditions of osmotic stress (sucrose, 17.6%, 72 hours)

Species	Biomass growth, % to control		Relative water content, % to control	
	Shoot	Root	Shoot	Root
T. monococcum L.	72.7±1.8**	85.7±2.5**	47.9±2.2**	77.4±2.0**
T. dicoccum Schuebl	90.0±2.2*	111.1±2.8*	76.2±3.8**	106.3±2.8
T. polonicum L.	85.7±2.8**	109.1±2.7*	70.5±3.6**	101.9±2.3
T. aethiopicum Jakubz.	90.3±2.5*	90.0±2.5**	75.3±3.8**	83.0±2.0**
T. compactum Host.	100.0±2.3	120.0±3.0**	88.1±4.2	112.5±2.8**
T. aestivum L.	68.4±1.9**	66.7±1.6**	55.5±2.5**	63.1±1.8**

*Characters * and ** indicate the accuracy of the t-test at 0.05 and 0.01 levels of significance, respectively*

conditions of osmotic stress were characterized by *T. compactum* Host. (88.1%), *T. dicoccum* Schuebl. (76.2), and *T. aethiopicum* Jakubz. (70.5%). The lowest relative water content of shoots and roots under osmotic stress was observed in *T. monococcum* L. (47.9% and 77.4%, respectively) and *T. aestivum* L. (55.5% and 63.1%, respectively).

It was revealed that all studied types of wheat differed in terms of the number of primary roots and the probability of roots, both under control and stressful conditions. The data are shown in Table 3.

Thus, stressful conditions had a slight impact on the number of primary roots. Only for the species *T. monococsum* L. and *T. compactum* Host. was there a significant decrease in this parameter with respect to the control (91.5% and 86.5%, respectively).

The maximum value of the root security parameter under stressful conditions with respect to the type of control was observed in *T. dicoccum* Schuebl. (95%). The minimum value was observed in *T. monococsum* L. (88.2%).

Since the effects of stress, primarily manifested in reduction in growth characteristics, the significant slowing in a plant's body height under adverse conditions can be caused, first of all, by the action of osmotic stress in zones of division and growth of the root-tip [33]. Extensive changes were induced by osmotic stress in the organization of most of the root-tip cells of wheat. Therefore, essential species-specific differences in the cytologic manifestations of the actions of osmotic stress on the cells of primary roots were noted.

Thus, the species of *T. monococcum* L. under stressful conditions demonstrated plasmolysis of the root-tip cells and vacuolation of the root cells (Fig. 1).

Under stressful conditions, the species of *T. dicoccum* Schuebl. showed that the cells were alive and their turgor was normal (Fig. 2).

Under osmotic stress, the root cells of *T. polonicum* L. demonstrated strong plasmolysis. Cells were elongated and had a small nucleus. Massive vacuolization and occasional fragmentation of the nucleus were noted (Fig. 3).

Table 3. The root security of various species of wheat plantlets under osmotic stress (sucrose, 17.6%, 72 hours)

Species	The number of primary roots, % to control	Root security, % to control		
		Control	Stress	% to control
T. monococcum L.	91.5±2.3*	37.8±3.8	33.3±3.7	88.2±2.2**
T. dicoccum Schuebl	94.9±2.6	25.8±2.3	24.5±2.3	95.0±2.6
T. polonicum L.	98.4±2.4	30.5±3.0	27.9±2.4	91.5±2.3**
T. aethiopicum Jakubz.	96.6±2.5	19.0±1.9	17.2±2.2	90.4±2.2**
T. compactum Host.	86.5±2.1**	20.3±2.2	22.7±2.4	89.4±2.4**
T. aestivum L.	96.6±2.5	30.8±3.0	21.1±2.3*	88.4±2.3**

*Characters * and ** indicate the accuracy of the t-test at 0.05 and 0.01 levels of significance, respectively*

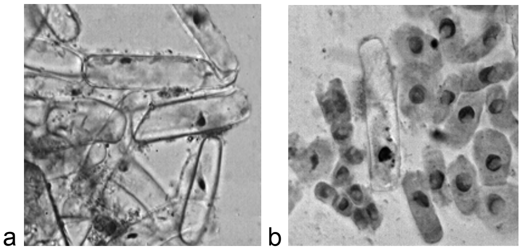

Fig. 1. The influence of osmotic stress on the root cells of *T. monococcum* L., where a – control, b – stress (sucrose, 17.6%, 72 hours), magnified ×40

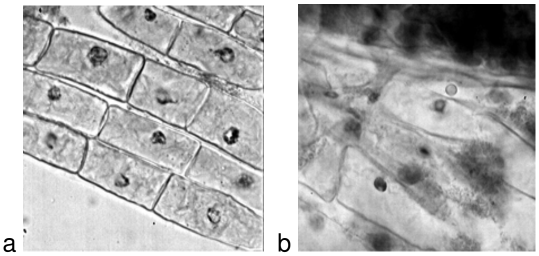

Fig. 2. The effect of osmotic stress on the root cells of *T. dicoccum* Schuebl., where a – control, b – stress (sucrose, 17.6%, 72 hours), magnified ×40

Under conditions of osmotic stress, *T. aethiopicum* Jakubz. exhibited small effects of plasmolysis; moreover, the root hairs and the formation of conductive vessels in a root were noted (Fig. 4).

In the cells of the root cap of species of *T. compactum* Host., plasmolysis was strong (Fig. 5).

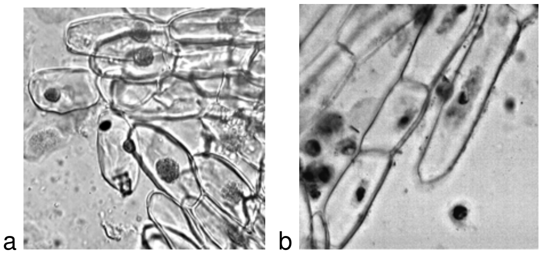

Fig. 3. The effect of osmotic stress on the root-tip cells of *T. polonicum* L., where a – control, b – stress (sucrose, 17.6%, 72 hours), magnified ×40

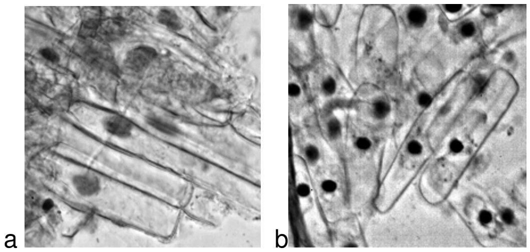

Fig. 4. The effect of osmotic stress on the root-tip cells of *T. aethiopicum* Jakubz., where a – control, b – stress (sucrose, 17.6%, 72 hours), magnified ×40

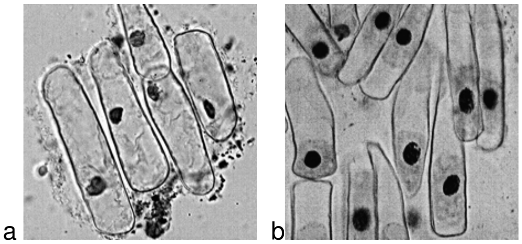

Fig. 5. The effect of osmotic stress on the root-tip cells of *T. compactum* Host., where a – control, b – stress (sucrose, 17.6%, 72 hours), magnified ×40

In the root cells, vacuolization, as well as the nuclear fragmentation and lysis of cell membranes were strong (Fig. 6).

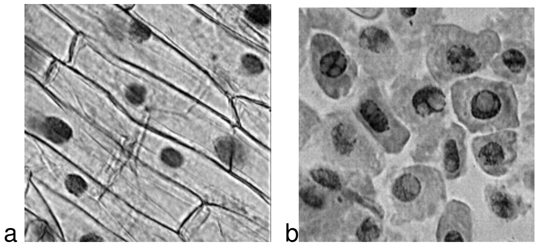

Fig. 6. The effect of osmotic stress on the root cells of *T. compactum* Host., where a – control, b – stress (sucrose, 17.6%, 72 hours), magnified ×40

The root cells of *T. aestivum* L. under osmotic stress were alive and able to divide. Root fibrils were observed. However, in the root hair and root cap cells, the developmental process of plasmolysis was observed (Fig. 7).

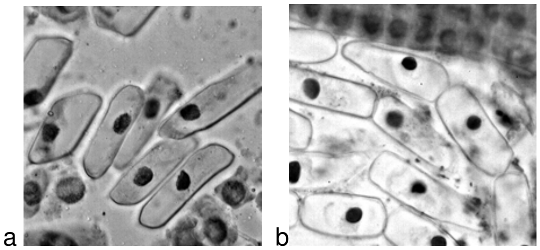

Fig. 7. The effect of osmotic stress on the root-tip cells of *T. aestivum* L., where a – control, b – stress (sucrose, 17.6%, 72 hours), magnified ×40

Blurring of the cytoplasm, the appearance of coarse sediments, and an increase in the cell penetration of various dyes was sometimes observed. The cytoplasm was compressed, indicating that the development process of plasmolysis had occurred – i.e., there were degenerative changes in the cellular structures (such as paranecrosis).

Overall, the degree of a plasmolysis varied between various types of cells, and also among cells from the different parts of a root. This was in accordance with Komis et al.'s data [34]. In the more stress-resistant forms of wheat, plasmolysis developed to a lesser extent under conditions of osmotic stress.

The phenomenon of paranecrosis is reversible. The root system of stable forms of wheat are less defective under stress. They are able to restore function at water supply restitution. However, if the effects of stress affect not only the water content, but also on the chromosomal apparatus of cells, the plant may die. This leads to the destruction of cells, such as through nuclear fragmentation. A prerequisite for fragmentation might be the vacuolization of the cell nucleus. Such pathologies that occur in stressful conditions can be observed in less stable forms of the plant.

It is believed that the productivity of crops is determined, first, by the volume of the root system, which correlates with the grain size [13]. The rooting depth and density are among the main drought avoidance traits identified that can confer seed yield in plants under terminal drought environments [13,35]. It is obvious that features of the root systems of plants are genetically determined. It is quite evident that the characteristics of the root system of plants are genetically determined. Their absorption and metabolic functions carry information not only about the productivity of plants, but also on their responses to stress [36]. In the field, seeds (due to a high suction force) germinate using winter moisture reserves. However, the lack of precipitation and the fast draining of soil may cause the death of the young plant in the future. The role of root morphology and anatomy in the overall root water transport capacity cannot be underestimated [37]. For grain cereals to efficiently extract moisture and avoid death in stressful conditions at the initial stages of their body height growth, a fast rate of rooting and a large number of germinal roots lead to normal development, even when there is a lack of moisture. In dry years, the vitality and productivity of the plants often depend exclusively on the germinal roots [38]. In rainfed environments, the depth of rooting is often cited as an important criterion because it has a major influence in determining the potential supply of water from the deep soil and thus improves yield [39,40]. This indicates the importance of prolific and deep root systems in maintaining the temperature regime of plants, which is perhaps due to water extraction by deep rooting [41].

Reducing the linear parameters of root growth under stress is the strategy that is used to reduce the flow of water from the roots into the soil while the soil's osmotic potential becomes lower than that of the roots [42]. However, soil drying does not occur at the same rate at different depths, and the drying rate is more pronounced in the superficial soil layers than in the deeper ones. Thus, plants that are able to develop a deeper root system are usually more tolerant to drought than plants with a more superficial root system. Thus, plants that are able to develop a deeper root system are usually more tolerant to drought than plants with a more superficial root system [43,44]. If the plants during germination have a greater number of roots, and the roots penetrate to a greater depth, these plants will faster move on to the autotrophic nutrition and be more productive. Although the linear growth parameters decrease upon root exposure to drought, under some specific circumstances of drought, an increase in root length has been reported [45,46]. However, the drought-sensitive forms of plants sometimes have higher root and shoot biomasses than the tolerant forms during the same soil water regimes under conditions of stress, which results in greater maintenance respiration and carbohydrate utilization [47]. These discrepancies could be caused by the different strategies that are used to overcome drought stress by the different plant species or cultivars. It should be noted that the signals (hydraulic or chemical) that regulate linear growth behavior under drought conditions are still unknown [14].

Within the tribe *Triticeae*, separate wheat species deserve special attention because they can be crossed with cultivars as sources of resistance to osmotic and salt stress. It is important to identify the most stable forms and their growth characteristics, regardless of the changes to the environmental water regime. In the experiments described above, it was shown that *T. dicoccum* Schuebl. and *T. aethiopicum* Jakubz – unlike the other species studied under conditions of osmotic stress – maintain high linear growth and root development parameters in conditions of water scarcity. Maintaining good water balance among the root system allows these plants to maintain high water content in the leaves. Preservation of these types of physiological activities under drought conditions might explain the active workings of the root system. Therefore, wheat species such as *T. dicoccum* Schuebl. and *T. aethiopicum* Jakubz have the most stable indicators for the development of root systems under conditions of osmotic stress; these plants can serve as valuable sources of these parameters, and they can be crossed with cultivated varieties of wheat to improve drought resistance among modern varieties.

4. CONCLUSION

Thus, a result of the experiments shows a clearly expressed common nonspecific cytological responses of cells of the root system of cereals to osmotic stress - reduction of linear growth parameters, plasmolysis. At the same time are marked a variety of specific changes, which depend on the genotype of the test forms. The nonspecific response processes of the cells of the root system were revealed and studied in various species of wheat under osmotic stress. As a result, we can recommend the use of certain methods to assess drought resistance among any species or variety of wheat in the early stages of ontogenesis.

The degree of species specificity was studied in response to osmotic stress; as such, it was possible to identify the plant forms that were tolerant to a lack of moisture in the early stages of ontogenesis. Wheat species such as *T. dicoccum* Schuebl. and *T. aethiopicum* Jakubz had the most stable indicators for the development of the root system of seedlings under osmotic stress. These species can serve as valuable sources of these parameters, and we can cross them with cultivated varieties of wheat to improve drought resistance among modern varieties.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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