

CHANGE IN LEAF ANATOMICAL PARAMETERS OF SEEDLINGS OF DIFFERENT WHEAT SPECIES UNDER CONDITIONS OF DROUGHT AND SALT STRESS

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Abstract

There were studied the anatomic parameters of leaves of 10-day-old seedlings of 7 different species of wheat after 72 hours of drought or salt stress and identified key areas of adaptation juvenile leaf tissue to adverse environmental factors. To detect differences between species and between groups of species differing in ploidy level there were identified anatomical features of the structure of each of the leaves of the studied species, grown under normal conditions and under the action of osmotic and salt stress. There were studied anatomical parameters of inner and external surface of the leaf. It was revealed that osmotic stress has an impact on the studied parameters of leaves of all the studied species. Analyzing the data, we can speak of a high adaptive capacity of tetraploid wheat species *T. dicoccum* Shuebl., *T. polonicum* L. and *T. aethiopicum* Jakubz. virtually all considered the anatomical parameters of the leaves. The results of this study show that indicators such as the increase in stress dimensions of protective and mechanical tissue and the mesophyll appear to be good criteria for selection of stress resistant forms of wheat.

Key words: Wheat, Species, Drought, Salt stress, Leaves, Anatomy.

Introduction

The question of the effect on the plant abiotic stressors such as drought and salinity, not only did not lose its importance, but is also becoming more urgent. The constant growth of the human population and the deterioration of the ecological balance of the planet is the need to intensify research in the field of vulnerability, adaptive capacity of plants under adverse conditions to improve production efficiency of the most important crops and above all - of wheat (Gupta, 2009; El-Shafey *et al.*, 2009; Mafakheri *et al.*, 2010).

However, with all the richness and diversity of the literature on wheat, there is little information on the structure of the photosynthetic tissue and quantitative anatomy of the tribe *Triticeae*. Studies devoted to the identification of resource potential and the laws of inheritance of wheat resistance to osmotic and salt stress fragmentary. Until now, there was little explored about structural features of phototrophic leaf tissues in species from different origins wheat genome (Hramtsova, 2004). Literature data indicate that the tetraploid species in this respect are the least studied, although the diversity and distribution area are widely presented (Shikhmuradov, 2014).

It is well known that the economic loss of the crop is greatest if the plant was subjected to stress in the juvenile stage (Rakhmankulova, 2012). But as far as we know, there are few reports about the effects of different level water stress on photosynthetic and metabolites of wheat seedlings.

Efficient diagnosis of wild wheat species and species with a limited economic value, including in the juvenile stage of development, makes it possible to define a more complete picture of their possible use in breeding and genetic programs and ways to preserve biodiversity. In this regard, anatomical and morphological studies remain relevant to assess the degree of adaptation of the plant as a whole, and its individual organs to changing environmental conditions (Mokronosov, 1978; Burundukova *et al.*, 2008).

This work is part of the comprehensive studies conducted by the Institute of Plant Biology and Biotechnology SC of the Ministry of Education and

Science to study the collection of wheat congeners, including various physiological aspects of their resistance to abiotic stresses for effective involvement in breeding and genetic programs as the starting material for interspecific crosses.

The main objective of the study is to quantify the anatomic parameters of leaves of 10-day-old seedlings of different wheat species after 72 hours of salt or drought stress and to identify key areas of adaptation juvenile leaf tissue to adverse environmental factors.

Materials and Methods

Seven winter wheat species adapted to the conditions of Kazakhstan, were used in the study: *T. monococcum*. (A^uA^u), *T. dicoccum* Schuebl. var. *atratum* (Host) Koern (A^uA^uBB), *T. polonicum* L. var. *villosum* (A^uA^uBB), *T. aethiopicum* Jakubz. (*T. abyssinicum* Vav.). (A^uA^uBB), *T. compactum* Host. (A^uA^uBBDD), *T. macha* ssp. *densiusculum* Dekapr. et. Menabde. (A^uA^uBBDD), *Triticum aestivum* L. (A^uA^uBBDD) – variety Saratovskaya-29. The choice of these facilities for research due to their differences in the genomic composition, as well as differences in terms of drought and salt tolerance, we have identified these forms previously (Terletskaia *et al.*, 2011; Terletskaia & Khailenko, 2015).

Seeds of plants of different wheat species were germinated in a growing chamber at 25°C. After 48 h, plantlets were transferred to 0.5L pots and were grown for 5 days in water culture. Then, for 72 hours, they were exposed to drought stress (17.6% sucrose solution) or salt stress (1.68% NaCl solution). Control plantlets were grown in water.

Anatomical analysis: Conservation of plants was carried out by the method of Strasburger-Flemming (Prozina, 1960). Fixation was performed in 70% ethyl alcohol. Preservative fluid is a mixture of alcohol-glycerol-water in a ratio of 1:1:1. Anatomical specimens were prepared with a microtome having a freezing unit TOC-2. Sections were placed in glycerine and balsam in accordance with

conventional techniques of Prozina (1960), Permyakova (1988) and Barykina (2004). The thickness of the anatomical sections was 10 to 15 microns. Micrographs of anatomic sections were made on a microscope with a camera MC 300 CAM V400/1.3M.

Statistical analysis: Samples for anatomical analysis were means of three samples for each treatment. The data of the experiment were analyzed statistically using two-way analysis of variance (ANOVA), with varieties and treatments as main effects of anatomical parameters. In this paper tables the characters * and ** show the accuracy at 0.05 and 0.01 levels of significance, respectively.

Results and Discussion

A cross section of the leaf blades of wheat seedlings *T. monococcum* L. (Fig. 1) shows, that from the outside the leaf is covered with epidermis. Among cells in the epidermis there can be distinguished larger cells called motor cells. Trichomes are seen on the epidermis. Between the upper and lower epidermis there is a tissue that consists of cells containing chlorophyll. This is assimilation parenchyma or mesophyll. Mesophyll is the main leaf tissue, which concentrates all the chloroplasts and photosynthesis. Mesophyll of this wheat is isospongy, the entire mesophyll of leaf consists of spongy cells. Mesophyll cells were fairly uniform in shape and structure, rounded, slightly elongated, with spikes, rather loosely coupled, between them there is the

intercellular space. In the mesophyll cells at some distance from each other are fibrovascular bundles. In wheat a lot about different size veins run along the sheet parallel to each other. On *T. monococcum* L. midrib vascular bundle is large, closed-collateral, xylem is directed to the upper epidermis and phloem to the bottom. Vascular bundle has a liner, barely containing chlorophyll. Above vascular bundle under the upper epidermis there are cells of mechanical tissue, sclerenchyma.

Figure 2 shows that the leaves of wheat seedlings of species *T. dicoccum* Shuebl. adaxial epidermal cells differ from the cells of the abaxial epidermis with more uniform shape and a rounded structure. As the axial and in the axial epidermis marked trichomes. Mesophyll leaf consists of a thin-walled assimilating tissue which comprises chloroplasts. The cells lining the vascular bundles are identical in form, large and fully isolate vascular bundle. Collateral vascular bundles, closed central midribs xylem consists of 2-3 vessels. Above and below the beams there is a mechanical tissue consisting of sclerenchyma cells. It is known that well developed sclerenchyma of leaves helps to reduce the harmful effects of drought (Ionova & Alabushev 2009).

During the study of of leaf blade of *T. polonicum* L. (Fig. 3) there was revealed the good development of the motor cells of the lower epidermis. They are located mainly between the veins. On both sides of the leaf blade there are clearly visible trichomes. In the central vein-a major vascular bundle with a facing around the vascular bundles there is mechanical cloth consisting of fibers of sclerenchyma.

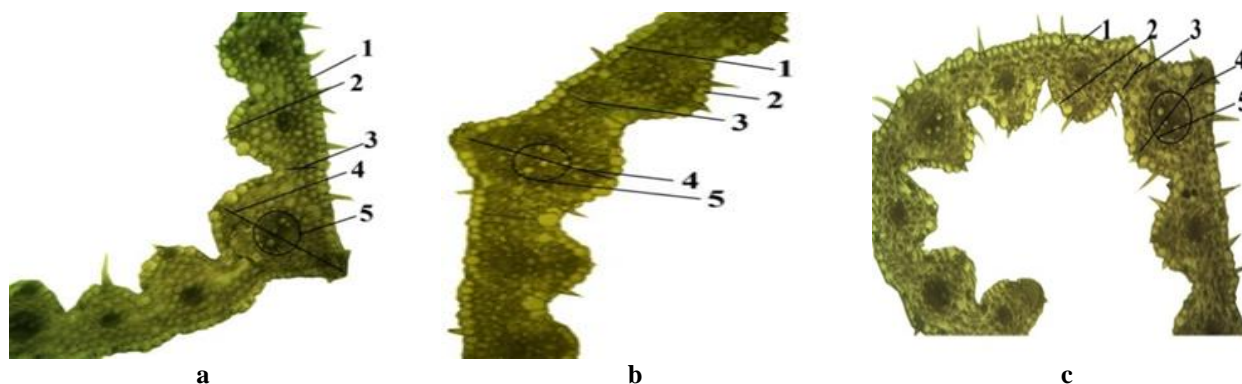


Fig. 1. Anatomical sections of the first leaf of *T. monococcum* L.
1. abaxial epidermis, 2. adaxial epidermis, 3. mesophyll, 4. central vascular bundle, 5. central vein
a. the control variant, b. drought stress, c. salt stress, increased x 100

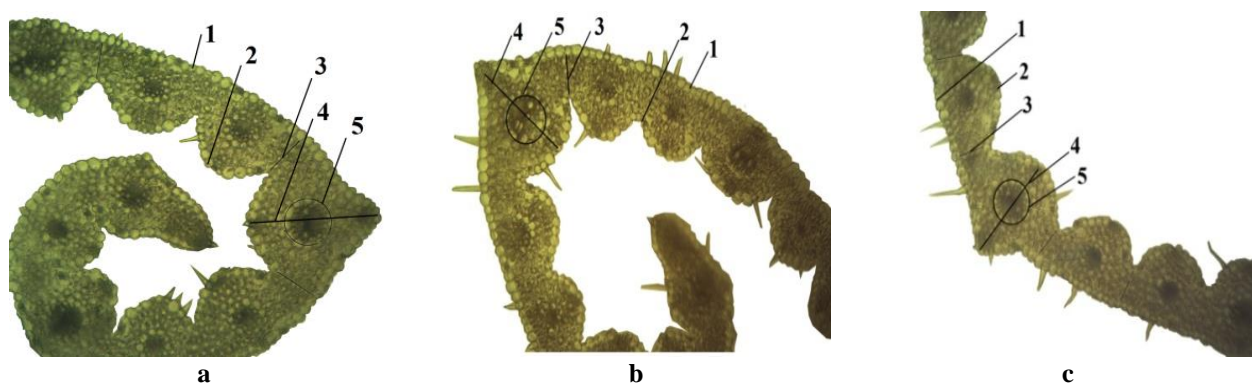


Fig. 2. Anatomical sections of the first sheet of *T. dicoccum* Shuebl.
1. abaxial epidermis, 2. adaxial epidermis, 3. mesophyll, 4. central vascular bundle, 5. central vein
a. the control variant, b. drought stress, c. salt stress, increased x 100

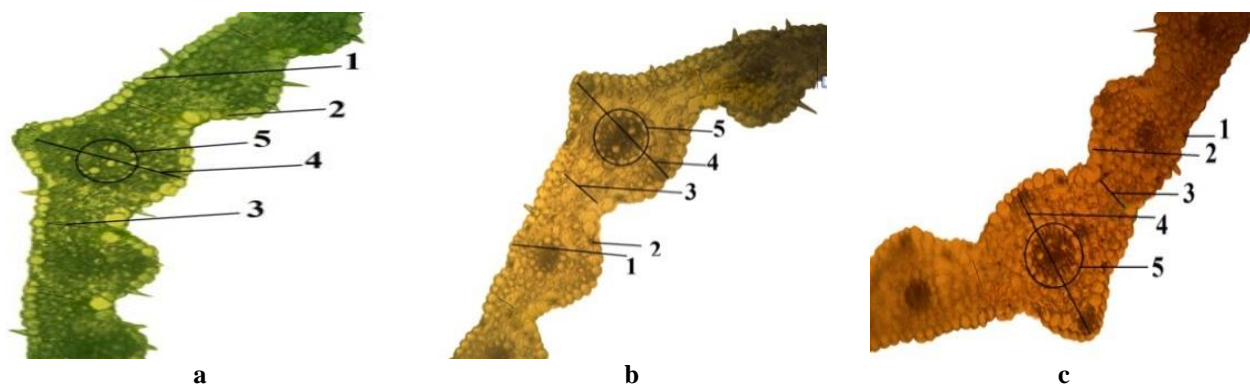


Fig. 3. Anatomical sections of the first leaf of *T. polonicum* L.

1. abaxial epidermis, 2. adaxial epidermis, 3. mesophyll, 4. central vascular bundle, 5. central vein
a. the control variant, b. drought stress, c. salt stress, increased x 100

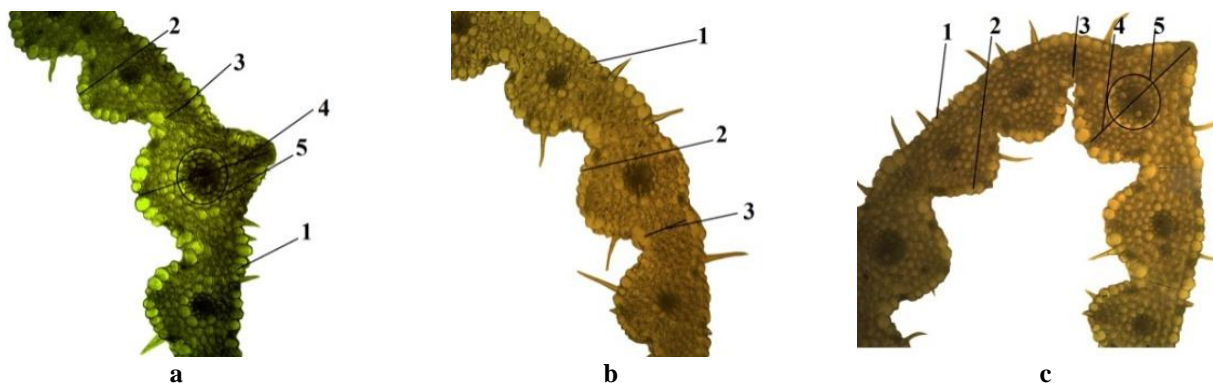


Fig. 4. Anatomical sections of the first sheet of *T. aethiopicum* Jakubz.

1. abaxial epidermis, 2. adaxial epidermis, 3. mesophyll, 4. central vascular bundle, 5. central vein
a. the control variant, b. drought stress, c. salt stress, increased x 100

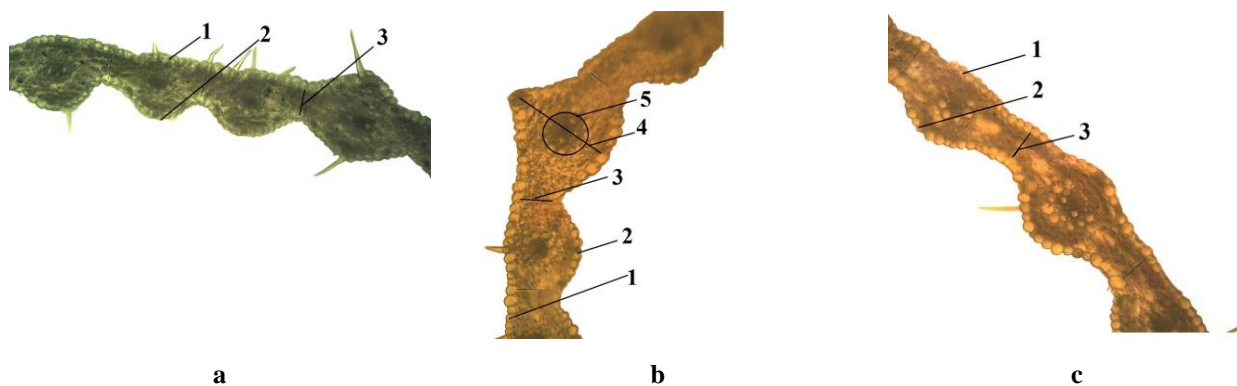


Fig. 5. Anatomical sections of the first leaf of *T. compactum* host.

1. abaxial epidermis, 2. adaxial epidermis, 3. mesophyll, 4. central vascular bundle, 5. central vein
a. the control variant, b. drought stress, c. salt stress, increased x 100

From the data presented in Fig. 4, it can be seen that in the species *T. aethiopicum* Jakubz. the leaf blade on both sides is covered with well-defined trichomes, cell size of the upper and lower epidermis is clearly visible, large cells. Between the veins on the upper and lower side of the leaf blade there can be detected motor cells. Conductive plates with beams, large, the central vein, they consist of 3 xylem vessels.

In accordance with Fig. 4, in the control variant of *T. aethiopicum* Jakubz. mesophyll cells and the lining did not have the strict order in positioning. In the central vein of parenchymal cells lining is bright and located in 2-3 rows unlike the other options, which lining the beams consisted of a single row of parenchymal cells.

Fig. 5 shows the anatomical sections of leaves of *T. compactum* host seedlings.

It is seen that in the species *T. compactum* Host. trichomes prevail in the adaxial epidermis. It was revealed that drought stress significantly affects the size of mesophyll cell species *T. compactum* Host.

During the study of the anatomic slices of leaf blades of seedling species *T. macha* Dek.et.Men. (Fig. 6), we have also indicated a significant effect of drought and salt stress on the development of leaf blades, in particular, the thickness of mesophyll and diameter of the central conductive beam.

The anatomical structure of the first leaf of *T. aestivum* L. seedlings is shown in Fig. 7.

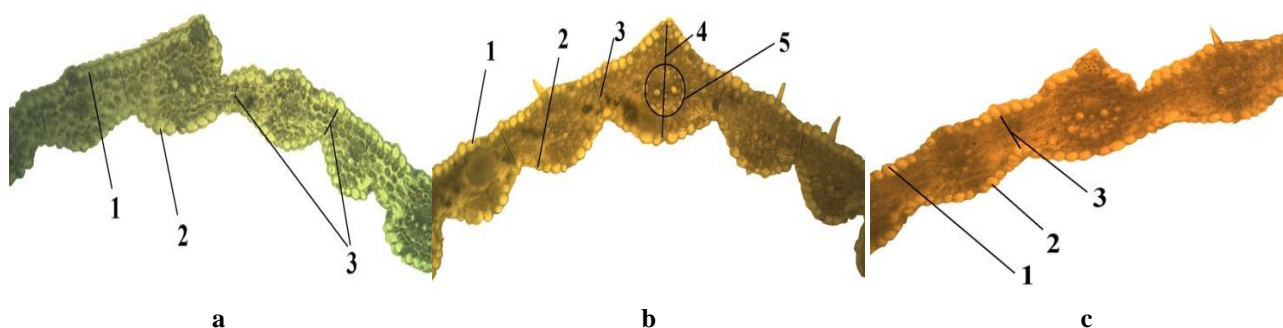


Fig. 6. Anatomical sections of the first leaf of *T. macha* Dek.et.Men.

1. abaxial epidermis, 2. adaxial epidermis, 3. mesophyll, 4. central vascular bundle, 5. central vein
a. the control variant, b. drought stress, c. salt stress, increased x 100

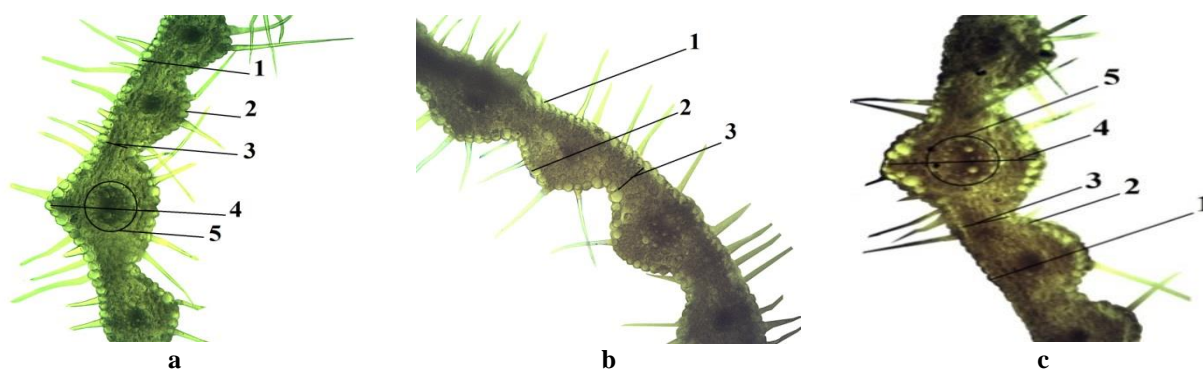


Fig. 7. Anatomical sections of the first leaf of *T. aestivum* L.

1. abaxial epidermis, 2. adaxial epidermis, 3. mesophyll, 4. central vascular bundle, 5. central vein
a. the control variant, b. drought stress, c. salt stress, increased x 100

There were noted expressed trichomes on both adaxial and abaxial leaf epidermis. On top of the large vascular bundle in the central vein there is well developed mechanical tissue consisting of sclerenchyma cells. The vascular bundles can be seen clearly, the xylem consists of three xylem vessels and in the two vessels there can be seen protoxylem lacunae. Unlike other kinds of wheat from *T. aestivum* L. there can be seen clearly distinguishable motor cells of the upper epidermis.

To detect differences between species and between groups of species differing in ploidy level there were identified anatomical features of each leaf structure of the studied species, grown under normal conditions and under the action of osmotic and salt stress.

Anatomical parameters of inner leaf surface:

Morphometric measurements of parameters of the inner surface of the leaf of wheat seedlings of different species under normal and stress conditions are presented in Table 1.

It was revealed that osmotic stress has an impact on the studied parameters of leaves of all the studied species.

Usually, the increase or decrease in value of a trait depends on the susceptibility to stresses.

The effect of tested abiotic stresses on the growth of leaves of wheat seedlings of different species as a whole is similar to that presented in the literature, its influence for soft wheat, barley and corn. This is a significant change (in the direction of increasing and decreasing) of the thickness of the abaxial and adaxial epidermal diameter vascular bundles of leaves as well as a decrease in stress conditions and the size of the diameter of the

stoma (Cárcamo *et al.*, 2012; Atabayeva *et al.*, 2013; Shireen *et al.*, 2015).

From the works of AT Mokronosov (1981) it is known that adaptation of photosynthesis to the action of environmental factors is carried out through the system of structural and functional rearrangements at different levels of the organization of the photosynthetic apparatus. Thick epidermis is known to be useful in checking water loss under limited moisture conditions along with the thickness of cuticle layers (Parveen *et al.*, 2001; Bahaji *et al.*, 2002). Thicker epidermis is expected to be adapted to harsh water limited environments like drought and salinity. Larger cortical cell area seems to be related to better storage of moisture that is essential for survival under harsh climates.

From the data presented in the Table 1 follows that the greatest thickening both abaxial and adaxial epidermis under stress conditions characterized species *T. dicoccum* Shuebl., *T. polonicum* L., *T. aethiopicum* Jakubz.

A similar arrangement of mesophyll cells allows us to characterize the type of structure leaf mesophyll all studied cereals as a loose cellular-isolateral-palisade. This structure combines features of mesophyll resistance to adverse environmental conditions and the possibility of a saturated metabolism (Dashtoyan, 2009). Mesophyll of wheat has heterogeneous composition of the cell population. Form, cell size change depending on external conditions, which implies the existence of an appropriate mechanism adaptation, directed at optimizing the structural and functional organization of the assimilation apparatus of leaves. The specificity of the structural and

functional changes of mesophyll depending on the lighting, temperature, humidity and other growth conditions (Mokronosov, 1978; Goryshina, 1989). Bastias *et al.* (2005), observing the decrease in the thickness of a leaf of maize under salt stress, there was noted the decrease in the number of cells and the size of mesophyll, indicating a limitation of cell growth. Therefore, increasing the thickness of the mesophyll under stressful conditions can serve as an indicator of stress. The cells are relatively large, they are less tightly packed, thereby, a larger volume of internal assimilation leaf surface persists. What is characteristic in our experiment for the species *T. aethiopicum* Jakubz. and *T. aestivum* L.

Veins or nerves act as water conductors and assimilate valves, moreover, they are mechanically strengthen the leaf (Shelepov *et al.*, 2004). In literature described that the thickness of midrib, interveinal distance and number of vascular bundles all progressively decreased with increasing salinity levels (Azmi & Alam, 1990).

In this experiment drought stress causes thickening of the central veins of almost all studied species except for wheat *T. aestivum* L. under salt stress a significant thickening of the central vein of the leaf was observed only in the species *T. polonicum* L. and *T. aethiopicum* Jakubz.

The water supply is the main function of major vascular bundles with well-developed xylem. Reducing the vascular bundles diameter is directly related to decreasing the area xylem vessels, which as conductive elements are clearly responsible for holding various elements by changing their diameter (Ortega *et al.*, 2006).

Munns (2002) indicated that the reduction of thickness and stem area might be because salt stress

reduces the ability of plants to take up water and this causes reduction in growth rate. If excessive amounts of salt enter the plant, they will eventually rise to toxic levels, transpiring leaves and reducing the photosynthetic capacity of the plant.

It is found that the drought-tolerant varieties of wheat leaf anatomically adapted not to reduce the outflow of nutrients from the leaves to the generative organs, and to increase the intensity of the outflow and increase transpiration process in comparison with drought-sensitive varieties. Drought-tolerant varieties form more powerful leaf conduction system under conditions of water stress and temperature (Ionova & Gaza, 2014). Greater vascular bundle size has been reported by Awasthi & Pathak (1999) and Iftikhar *et al.* (2009) in saline tolerant genotypes of *Ziziphus* species and *Eucalyptus* species, what perhaps indicating the better adaptation to a variety of environment types, and hence, the wider distributional range as compared to other species.

As follows from the data presented in Table 1, in most of the species except for *T. monococcum* L. *T. aestivum* L. and size of the central beam as a conductive during drought stress and under salt stress increases, which is indicative of its high adaptation ability to the tested abiotic stresses.

The literature sources noted that in the process of selection of the genus *Triticum* with increasing ploidy level of the nucleus, increased productivity was achieved by accelerating cell division and expansion, in which the size of the leaves were growing, and the total internal assimilation area was reduced (Zvereva, 2010).

Table 1. Morphometric parameters of the first leaves of different wheat species in control and abiotic stress conditions.

Species	The thickness of the adaxial epidermis	The thickness of the abaxial epidermis	The thickness of the mesophyll	The thickness of the central vein	Size of the central vascular bundle
Control					
<i>T. monococcum</i> (A ^u A ^u)	38.25 ± 2.50	31.50 ± 1.98	117.48 ± 17.80	490.57 ± 0.90*	39408.14*
<i>T. dicoccum</i> (A ^u A ^u BB)	37.25 ± 2.02	38.80 ± 2.01	158.22 ± 2.30*	480.61 ± 1.80*	32614.23*
<i>T. polonicum</i> (A ^u A ^u BB)	32.77 ± 0.65	26.57 ± 3.31	146.89 ± 0.98*	470.62 ± 3.40*	23235.22*
<i>T. aethiopicum</i> (A ^u A ^u BB)	39.07 ± 1.70	36.97 ± 2.10	117.34 ± 0.90*	490.57 ± 2.30*	39408.14*
<i>T. compactum</i> (A ^u A ^u BBDD)	32.60 ± 3.04	34.82 ± 2.60	105.73 ± 4.60	377.47 ± 7.80	11309.73
<i>T. macha</i> (A ^u A ^u BBDD)	36.86 ± 2.20	31.19 ± 2.70	87.37 ± 5.10	385.54 ± 4.40	13684.78
<i>T. aestivum</i> (A ^u A ^u BBDD)	36.65 ± 1.42	27.94 ± 1.40	106.18 ± 3.80	411.71 ± 2.30	33979.468
Drought stress (17.6% sucrose solution, 72h)					
<i>T. monococcum</i> (A ^u A ^u)	37.25 ± 1.70	32.75 ± 0.50	144.29 ± 0.60	481.36 ± 0.70	28952.92
<i>T. dicoccum</i> (A ^u A ^u BB)	42.25 ± 1.70*	40.05 ± 0.80*	145.25 ± 0.80	529.91 ± 1.90*	33575.91*
<i>T. polonicum</i> (A ^u A ^u BB)	43.80 ± 0.80*	39.37 ± 0.70*	145.23 ± 0.50	553.59 ± 6.20*	54739.11*
<i>T. aethiopicum</i> (A ^u A ^u BB)	41.80 ± 0.80*	39.37 ± 0.70*	135.23 ± 0.50	553.59 ± 6.20*	34739.22*
<i>T. compactum</i> (A ^u A ^u BBDD)	38.98 ± 0.20	35.47 ± 0.50	138.03 ± 2.90	488.26 ± 4.50	22167.12
<i>T. macha</i> (A ^u A ^u BBDD)	33.05 ± 1.30	35.86 ± 1.10	89.31 ± 3.80	421.54 ± 3.60	18145.84
<i>T. aestivum</i> (A ^u A ^u BBDD)	31.67 ± 2.80	28.94 ± 1.60	124.67 ± 0.70	374.99 ± 3.50	16361.22
Salt stress (1.68% NaCl solution, 72h)					
<i>T. monococcum</i> (A ^u A ^u)	40.20 ± 0.42*	30.86 ± 2.70	112.72 ± 3.30	446.93 ± 0.80	22268.91
<i>T. dicoccum</i> (A ^u A ^u BB)	39.69 ± 0.21*	38.60 ± 0.60*	129.71 ± 2.90*	489.57 ± 2.20*	31489.73
<i>T. polonicum</i> (A ^u A ^u BB)	41.44 ± 1.01*	48.24 ± 1.80*	125.89 ± 3.30*	556.35 ± 2.40*	42273.27*
<i>T. aethiopicum</i> (A ^u A ^u BB)	42.14 ± 1.01*	45.57 ± 1.80*	126.41 ± 2.70*	608.12 ± 0.90*	42273.27*
<i>T. compactum</i> (A ^u A ^u BBDD)	32.53 ± 2.20	22.69 ± 3.60	103.60 ± 1.80	347.91 ± 2.80	16228.02
<i>T. macha</i> (A ^u A ^u BBDD)	32.32 ± 1.10	29.93 ± 1.40	88.07 ± 0.90	372.72 ± 4.60	14933.72
<i>T. aestivum</i> (A ^u A ^u BBDD)	29.53 ± 2.20	22.69 ± 3.60	103.60 ± 1.80	356.91 ± 2.83	14228.89

Note: The characters * indicate the accuracy of the t-test at 0.05 levels of significance

A few recent data on the relationship between the intensity of photosynthesis, the parameters of the structure of the photosynthetic apparatus, the characteristics of growth and assimilation surface ploidy wheat say that:

1. Modern species of wheat with different numbers of chromosomes and genomic origin set are significantly different from the ancestral forms of the growth parameters, quantitative characteristics of the structure and leaf mesophyll photosynthetic activity.
2. The observed differences have arisen as a result of changes in ploidy and genomic structure of the nucleus in the process of evolution of the genus *Triticum* L. They are associated with the restructuring of the internal structure of phototrophic leaf tissue and changes in the functional activity of a single chloroplast.
3. Changes in the number and size of cells optimize the structure of phototrophic leaf tissue from tetra- and hexaploid wheat species. This leads to an increase in the inner surface of the assimilation and therefore, to the conductivity of the leaf to reduce the intensity and CO₂ photosynthesis in modern types of wheat compared to the ancestral forms (Hramtsova *et al.*, 2003).

In accordance with Table 1, it showed that under control conditions the mesophyll thickness, the thickness of the central vein leaves and the Size of the central vascular bundle at the di- and tetraploid species were higher than hexaploid.

Analyzing the data, we can speak of a high adaptive capacity of tetraploid wheat species *T. dicoccum* Shuebl., *T. polonicum* L. and *T. aestivum* Jakubz. virtually with all considered anatomical parameters of the leaves.

The anatomical parameters of external surface of leaves: A big role in the maintenance of the water balance and the regulation of the leaf temperature play trichomes, which also play a protective role with respect to the pathogen, promote pollination at the flowering stage and impact on photosynthesis (Farquhar *et al.*, 2002; Gudkova, 2013).

There was studied the distribution of trichomes in the adaxial and abaxial epidermis of leaf blade and the influence of the osmotic salt stress and change of the length of the trichomes of leaf blades of different types of wheat seedlings.

There were revealed species differences in the distribution of trichomes on the leaf surface (Table 2).

The largest number of trichomes per 1 mm² as the adaxial and abaxial epidermis with an average variation of characteristic values in the absence of stress observed in the species *T. monococcum* L. (44.9 ± 5.7 and 81.8 ± 3.7 respectively), *T. dicoccum* Shuebl. (53.0 ± 5.5 and 67.6 ± 4.8) and *T. aestivum* L. (42.0 ± 3.5 and 61.5 ± 4.0). At the same in all species, except for *T. aestivum* Jakubz. and *T. compactum* Host., in which the hairiness of the upper and lower surfaces of the leaf did not differ, had greater hairiness of abaxial epidermis.

There are shown species differences in trichome dine of adaxial and abaxial epidermis. There is noted an increase in the length of the trichomes with increasing ploidy wheat - the most long trichomes characterized hexaploid form of *T. aestivum* L., lowest - diploid type of *T. monococcum* L. (Fig. 8).

There is marked a change in leaf pubescence seedlings of all species under stress conditions (Table 3).

Table 2. The number of trichomes on the of leaf blade 1 mm² in 10-day-old seedlings of different wheat species.

Species	The number of trichomes per 1 mm ² of leaf blade			
	adaxial epidermis		abaxial epidermis	
	M ± m	C _v	M ± m	C _v
<i>T. monococcum</i> L.	44,9 ± 5,7	17,3	81,8 ± 6,7*	13,1
<i>T. dicoccum</i> Schuebl.	53,0 ± 5,5*	13,6	67,6 ± 5,8	11,9
<i>T. polonicum</i> L.	10,5 ± 2,6**	15,5	19,5 ± 3,6**	26,0
<i>T. aestivum</i> Jakubz.	34,5 ± 5,2	18,8	39,9 ± 4,2*	15,9
<i>T. compactum</i> Host.	21,2 ± 3,8**	16,3	16,7 ± 3,2**	14,7
<i>T. aestivum</i> L.	42,0 ± 4,5	11,5	61,5 ± 5,7	12,6

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

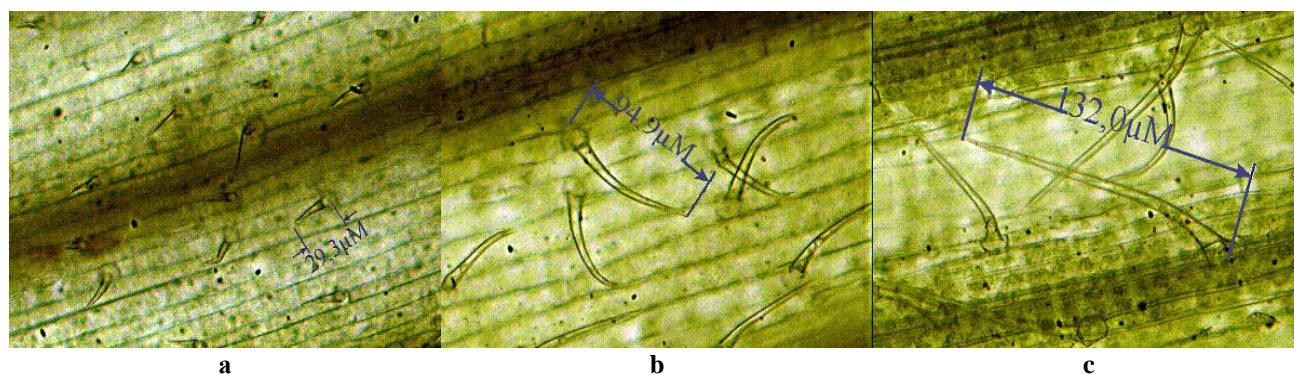


Fig. 8. The differences in trichomes length depending on the ploidy of wheat species, increase x 10. a. *T. monococcum* L. (A^uA^u), b. *T. aestivum* Jakubz. (A^uA^uBB), c. *T. aestivum* L. (A^uA^uBBDD)

Table 3. Change the size of trichomes of leaf blades of 10-day-old seedlings of different species of wheat depending on the influence of stress factors.

Species	The length of the trichomes, μm					
	Adaxial epidermis			Abaxial epidermis		
	Control	Drought stress (17.6% sucrose solution, 72 h)	Salt stress (NaCl, 1,68%, 72h)	Control	Drought stress (17.6% sucrose solution, 72 h)	Salt stress (NaCl, 1,68%, 72h)
<i>T. monococcum</i> L.	29.3 \pm 1.6	23.3 \pm 1.2*	25.9 \pm 1.0	30.1 \pm 1.7	30.8 \pm 1.5	27.8 \pm 1.2
<i>T. dicoccum</i> Schuebl.	85.9 \pm 3.1	79.1 \pm 2.9	75.8 \pm 2.6*	79.9 \pm 2.9	78.6 \pm 2.8	69.8 \pm 2.6*
<i>T. polonicum</i> L.	34.9 \pm 2.5	36.0 \pm 2.7	31.5 \pm 2.4	41.3 \pm 1.9	50.6 \pm 1.7*	45.0 \pm 1.6
<i>T.aethiopicum</i> Jakubz.	94.9 \pm 3.4	98.6 \pm 2.6	90.8 \pm 3.1	83.6 \pm 2.5	86.6 \pm 2.8	92.6 \pm 2.5*
<i>T. compactum</i> Host.	58.9 \pm 2.7	77.3 \pm 2.4**	57.8 \pm 2.4	65.3 \pm 2.4	69.4 \pm 2.1	65.3 \pm 2.2
<i>T. aestivum</i> L.	132.0 \pm 3.6	148.9 \pm 3.8*	193.9 \pm 4.4	163.9 \pm 4.2	122.3 \pm 3.6**	186.8 \pm 4.2*

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

Table 4. The number of stomata per 1 mm² of leaf blade in 10-day-old sprouts of different wheat species.

Species	The number of stomata per 1 mm ² of leaf blade			
	Adaxial epidermis		Abaxial epidermis	
	M \pm m	C _v	M \pm m	C _v
<i>T. monococcum</i> L. (A ^u A ^u)	90.9 \pm 5.0**	6.9	62.7 \pm 4.3**	7.1
<i>T. dicoccum</i> Schuebl. (A ^u A ^u BB)	43.0 \pm 2.5*	3.4	40.0 \pm 3.5*	7.4
<i>T. polonicum</i> L. (A ^u A ^u BB)	41.5 \pm 3.2*	5.9	30.2 \pm 2.9*	6.7
<i>T. aethiopicum</i> Jakubz. (A ^u A ^u BB)	50.5 \pm 3.5*	7.2	31.1 \pm 2.4*	4.5
<i>T. compactum</i> Host. (A ^u A ^u BBDD)	40.6 \pm 2.9*	4.9	25.5 \pm 1.9**	3.4
<i>T. aestivum</i> L. (A ^u A ^u BBDD)	58.9 \pm 3.8*	5.9	32.3 \pm 2.8*	5.8

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

Table 5. Change of the size of the stomatal apparatus of the adaxial epidermis of leaf blades of 10-day-old seedlings of different species of wheat depending on the influence of stress factors.

Species	Length of the stomata, μm			Width of the stomata, μm		
	Control	Drought stress (17.6% sucrose solution, 72 h)	Salt stress (NaCl, 1,68%, 72h)	Control	Drought stress (17.6% sucrose solution, 72 h)	Salt stress (NaCl, 1,68%, 72h)
	<i>T. monococcum</i> L.	39.0 \pm 2.6	39.0 \pm 2.6	36.8 \pm 1.4	19.5 \pm 1.9	18.4 \pm 1.5
<i>T. dicoccum</i> Schuebl.	52.9 \pm 1.8	51.4 \pm 1.9	49.9 \pm 1.0	19.4 \pm 0.9	20.3 \pm 0.8	19.1 \pm 0.6
<i>T. polonicum</i> L.	52.9 \pm 1.7	51.8 \pm 1.9	50.1 \pm 1.6	23.6 \pm 1.7	22.1 \pm 1.3	22.1 \pm 1.4
<i>T.aethiopicum</i> Jakubz.	53.3 \pm 2.9	51.8 \pm 1.6	52.1 \pm 2.4	20.3 \pm 0.7	20.6 \pm 0.8	21.0 \pm 1.5
<i>T. compactum</i> Host.	56.6 \pm 3.2	57.0 \pm 2.4	55.5 \pm 1.3	22.1 \pm 1.4	20.6 \pm 0.7	19.5 \pm 0.6
<i>T. aestivum</i> L.	72.4 \pm 2.9	67.1 \pm 2.7	63.8 \pm 2.8*	27.8 \pm 1.7	23.3 \pm 1.2*	22.5 \pm 1.2*

Note: the characters * indicate the accuracy of the t-test at 0.05 levels of significance

Under conditions of drought stress in species such as *T. aethiopicum* Jakubz., *T. compactum* Host. and *T. aestivum* L. there was noted tendency to increase the length of the adaxial trichomes, (and, in the species *T. compactum* Host., and *T. aestivum* L. difference was statistically significant) and the species *T. aethiopicum* Jakubz., *T. compactum* Host. - also of the abaxial leaf surface.

In species *T. monococcum* L. there was a statistically significant reduction in the length of the trichomes of adaxial epidermis under induced drought conditions and in the species *T. dicoccum* Shuebl. there was significantly reduced the growth of trichomes of abaxial and adaxial epidermis under salt stress.

Under salt stress conditions, the length of the trichomes of the adaxial epidermis increased only in species *T. aestivum* L. Trichomes of such species as *T. polonicum* L., *T. aethiopicum* Jakubz., *T. compactum* Host. and *T. aestivum* L. (abaxial epidermis) were not lowered or vice versa, increased growth. In species *T. compactum* Host. and *T. aestivum* L. the difference was statistically significant.

An important role in the control of leaf gas exchange between the atmosphere play stomata - the main "gateway" for the passage of water vapor, carbon dioxide and oxygen (Rebeille, 1988). There was discovered interconnection level of gas exchange with varying size of

stomata and their number per unit area of leaf cultivars (Pinheiro *et al.*, 2008), as well as a significant influence on the genetic component of variation in the density of stomata of wheat.

Experimentally we identified specific differences in the size and the number of stomata per unit of leaf blade area. Table 4 presents data on the number of stomata per 1 mm² of leaf blade in 10-day-old sprouts of different wheat species.

It is shown that the number of stomata per 1 mm² axial leaf epidermis of all the species is bigger than abaxial surface (from 40.6 \pm 3.9 to 90.9 \pm 7.0 and from 30.2 \pm 2.9 to 62.7 \pm 4.3 respectively). Variation characteristic values were low. There was a downward trend in the number of stomata per 1 mm² of leaf surface with increasing ploidy wheat. An exception is the kind of hexaploid *T. aestivum* L., in the number of stomata per unit area adaxial surface exceeds the tetraploid species.

The total area of stomata adaxial epidermal ranged from 5 to 12%, abaxial epidermis - 3 to 5% of leaf area (1 mm²) and *T. aestivum* L. species was also maximal. The dependence of the size of the stomata on ploidy wheat species and on the influence of the action of stress factors (Tables 5, 6).

Table 6. Change of the size of the stomatal apparatus of the abaxial epidermis of leaf blades of 10-day-old seedlings of various types of wheat depending on the influence of stress factors.

Species	Length of the stomata, μm			Width of the stomata, μm		
	Control	Drought stress (17.6% sucrose solution, 72 h)	Salt stress (NaCl, 1,68%, 72h)	Control	Drought stress (17.6% sucrose solution, 72 h)	Salt stress (NaCl, 1,68%, 72h)
<i>T. monococcum</i> L.	39.8 \pm 2.3	40.1 \pm 1.9	37.5 \pm 1.8	17.6 \pm 1.1	18.4 \pm 1.5	17.6 \pm 1.2
<i>T. dicoccum</i> Schuebl.	52.3 \pm 1.2	51.4 \pm 2.3	48.8 \pm 2.1	19.9 \pm 1.2	19.9 \pm 1.7	20.3 \pm 1.6
<i>T. polonicum</i> L.	52.5 \pm 1.7	52.1 \pm 3.9	52.4 \pm 2.2	23.3 \pm 1.5	22.9 \pm 1.4	22.4 \pm 1.8
<i>T.aethiopicum</i> Jakubz.	55.1 \pm 2.2	52.5 \pm 2.6	52.5 \pm 2.4	19.9 \pm 0.7	20.6 \pm 1.4	21.0 \pm 1.3
<i>T. compactum</i> Host.	54.0 \pm 1.3	55.5 \pm 3.5	55.9 \pm 2.1	20.6 \pm 0.7	19.9 \pm 0.6	19.5 \pm 0.5
<i>T. aestivum</i> L.	67.9 \pm 2.4	67.1 \pm 3.0	67.1 \pm 3.1	25.1 \pm 2.9	24.4 \pm 2.7	23.3 \pm 1.0

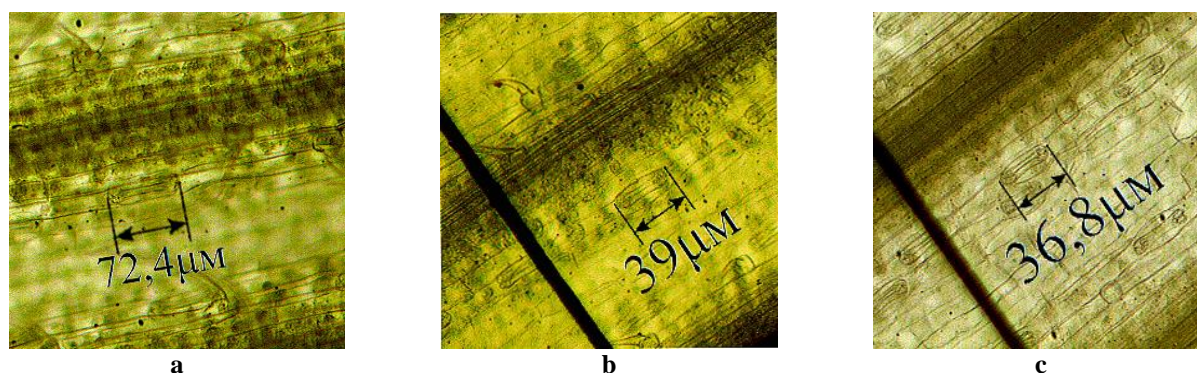


Fig. 9. Change of the length of stomata adaxial leaf surface, depending on the ploidy and the effects of stress a. *T. aestivum* L. (control), b. *T.monococcum* L. (control), c. *T.monococcum* L. (NaCl, 168%, 72h)

It is noted that an increase in ploidy increases the length of the stomata by 39.0 μm at *T. monococcum* L. to 72.4 μm at *T. aestivum* L. Especially there is a clear dependence on the length of stomata on the ploidy species under study expressed in the control variant of the experiment on the adaxial leaf surface (Fig. 9a, b, c).

It was revealed that the effect of the stressors not only leads to the closure of stomata, but also to their deformation - compression, bending, reducing linear value of the width and length or, alternatively, swelling "mucilaginated" from less stable forms, which causes a slight increase the linear values of the width. In stressful conditions was a significant decrease in the parameters of length and width of stomata adaxial epidermis species *T.aestivum* L.

Conclusion

Anatomical and morphological changes that allow plants to withstand the stress effects are mainly focused on maintaining the effectiveness of the use of water and carbon balance gain in resistance of plants to drought and salt. Therefore, changing the anatomical characteristics leaves under stress can be regarded as a significant manifestation of the regulation of photosynthesis at the morphological level, ensuring the optimization and adaptation of the photosynthetic apparatus.

The results of this study show that indicators such as the increase in stress dimensions of protective and mechanical tissue and the mesophyll appears to be good criteria for selection of stress resistant forms of wheat.

The study of anatomical parameters of leaves seedlings of different species of wheat helps to identify adaptive features that can guide the choice of plants for expansion work on the introduction and hybridization in arid climates.

As a result of the studies, a higher adaptive ability of tetraploid wheat species *T. dicoccum* Shuebl., *T. polonicum* L. and *T. aethiopicum* Jakubz. as found in comparison with hexaploid ones in practically all considered anatomical parameters of the leaf. Attention for future research deserve the data obtained on the basis of morphometric measurements of anatomical parameters of leaves under normal and stress conditions, tetraploid wheat species such as *T. dicoccum* Shuebl., *T. polonicum* L. and *T. aethiopicum* Jakubz. Thus, the data obtained from these species deserve much attention for future research.

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