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Morphophysiological and Photosynthetic Reactions of Wheat (*T. aestivum* L.) and Its Wild Congeners to Drought Condition In Vivo and In Vitro

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Abstract

The results of the complex analysis of different wheat species tolerance to drought stress in vivo at different levels of organization as well as in vitro on callus cultures, which was conducted, are presented. The objects of research are as follows: species of wheat—*T. monococcum* L. (A^u), *T. dicoccum* Shuebl. (A^uB), *T. polonicum* L. (A^uB), *T. aethiopicum* Jakubz. (A^uB), *T. macha* Dek.et.Men. (A^uBD), *T. compactum* Host. (A^uBD), and *T. aestivum* L. (A^uBD). The methods of this research are physiological, biotechnological, and cytological. The focus was on nonspecific reactions of cereals to osmotic stress, and species-specific changes according to growth and anatomical and photosynthetic parameters which depend on studied species ploidy were shown. It was shown that results of testing of plant tissues in vivo and in vitro are comparable. It demonstrated the criterions for selection of drought-tolerant forms. Optimal selection criteria were identified, and more drought-resistant wheat species were identified.

Keywords: wheat, species, leaves, callus, drought, morphophysiology, anatomy, photosynthesis

1. Introduction

According to the UN [1], desert or dry lands represent about half of terrestrial environments worldwide. Lack of water has become a key stressor. The continued growth of the human population and deterioration of the ecological balance necessitate intensifying research in plant vulnerability and adaptive capacity under adverse conditions to enhancing productivity of the most important crops and—above all—of wheat [2–4].

The understanding of how drought affects plants is an actual necessity now. Soil drought develops with a decrease in moisture reserves in the soil to a constant wilting. During atmospheric drought, water reserves in the soil may not reach a critical level, but the relative humidity of the air, which decreases to 30% and below, can

cause intense transpiration. The combination of soil and atmospheric droughts is particularly damaging to plants. Short-term, tough, “shock”-type impacts affect primarily the structural organization of the plant. Slowly increasing and long-term effects of an unfavorable factor affect the functional organization of the plant, the sphere of basal metabolism. The hardest effects are those vegetative and generative organs which are affected by drought from the beginning of their formation.

The genetic potential used in wheat breeding today is insufficient, and data on the nature of the drought tolerance of wild relatives of wheat may open up new possibilities for searching and creating promising breeding material. The tribe *Triticeae*, in which hard bread belongs, has great potential for stress tolerance. Wild members of the tribe, among which there are halophytes, grow in a wide range of conditions throughout the world and have large genetic variations [5, 6]. Effective diagnostics of wild-growing species of wheat and species of limited economic importance make it possible to form a more complete picture of their possible use in breeding and genetic programs and ways to preserve biodiversity [7]. The introduction of the new species with great potential constitutive adaptability into the culture (the direction of a “change of species”) along with the genetic potential of cultivated species requires intensive research owing to the multiple unsuccessful attempts to increase stress tolerance, maturation rate, and photosynthetic productivity of plants [8].

Currently, wild congeners of wheat and their derivative forms are most often considered as sources of disease and pest-resistant genes. Much less often, these species are involved in biochemical and morphophysiological studies [5]. Studies which are devoted to the identification of the resource potential and patterns of inheritance of wheat resistance to extreme stress are fragmentary. So far, there is very little information about the structure of photosynthetic tissue and the quantitative anatomy in species from different wheat species [9, 10]. Literature data indicate that the tetraploid species in this respect are the least studied, although the diversity and distribution area are widely presented [11]. Comparative studies of different wheat species for differences of morphologic and physiologic structures in both wild relatives and cultivated will contribute to obtaining new information to more effectively identify the limiting units of the production process.

The selection process involved factors of changes in the structure, size, and duration of the photosynthetic apparatus, but the activity of the photosynthetic apparatus remained at a level close to the original (Evans Paradox) [12, 13]. The few literature data on the relationship between the intensity of photosynthesis, parameters of the structure of the photosynthetic apparatus, growth characteristics of the assimilation surface, and the level of wheat ploidy suggest that modern types of wheat with different numbers of chromosomes and the origin of the genomic set are significantly different from the ancestor forms in terms of growth parameters, quantitative characteristics of the structure of the leaf mesophyll, and its photosynthetic activity. The observed differences arose as a result of changes in ploidy and the genomic composition of the nucleus during the evolution of the genus *Triticum* and are associated with rearrangements of the internal structure of the phototrophic leaf tissues and changes in the functional activity of a single chloroplast. The change in the number and size of cells optimizes the structure of phototrophic leaf tissues in tetra- and hexaploid wheat species, which leads to an increase in the internal assimilation surface and, consequently, leaf conductivity for CO₂ and a decrease in photosynthesis intensity in modern wheat species compared to ancestral forms and *Aegilops* species [14]. Therefore, studies aimed at finding plants with a developed and active photosynthetic apparatus for their involvement in the breeding process are also still very relevant [15–18].

Thus, it was shown that a high density of pubescence is characteristic of drought-resistant forms, and for diploid species the presence the genes of control of leaf pubescence was determined [18, 19]. Probably, each of the elements of the genome of polyploidy wheat has its own genetic control system of leaf pubescence. The wheat may have homoallelic genes, functionally degenerate or functionally complementary, which also increase the total number of genes involved in the control of this adaptive trait [20].

In the *Triticeae* tribe, diploid species are characterized by a shorter stomatal cell length compared with tetra- and hexaploid species [21]. Larger guard cells were found in spring wheat varieties (*Triticum aestivum* L.) and are associated with spring-type developmental alleles (Vrn-A1a), while the smallest ones are associated with developmental winter-type alleles (Vrn-A1b) on chromosome 5A.

The effect of chromosomes 1A, 3A, 4A, 5A, 1B, 5B, 6D, 7A, and 7D in different wheat varieties on the linear dimensions of stomata is also shown [6]. It was also noted that there is a significant correlation between the variation in the size and density of the stomata of wheat and its yield, both in normal and arid growing conditions [14]. Lamari found a significant effect of the genetic component on the variation in the density of the stomata of the wheat leaf [22].

It is known that the level of ploidy of plants, as an indicator related to cell size, is responsible for the thickness of the lamina [23]. The parameters of the leaf plates, determined by the genotype, are directly related to photosynthesis—negative correlations were found between the intensity of photosynthesis and the ratio of the mesophyll cell size to the ploidy level of the nuclear genome [24]. However, the size of the leaf surface, being a genetically determined trait, is largely corrected by specific environmental conditions.

Drought tolerance is a complex trait which is a combined function of various morphological (leaf emergence and flowering, coleoptile length, leaf area, leaf rolling, wax content, awns, stomatal density, root characteristics, cell membrane stability, etc.) [7], physiological (transpiration rate, water use efficiency, stomatal conductance, osmotic adjustment, relative water content, leaf turgor, etc.), and different biochemical characters [25].

Physiological functions of plants are closely related to their morphological and anatomic features. The dehydration of tissues which is arising during drought changes the course of physiological biochemical processes that in turn affects growth processes, anatomy, and morphology of plants. The lower leaves play an important role at the first stages of development, when the issue of survival of sprout in stressful conditions is actually resolved and the root system is created. Leaves of the upper layers (stem leaves) have crucial importance in photo assimilatory work of a plant, especially during of spike and grain formation—a critical period which is determining the size of losses of potential productivity in the conditions of insufficient water supply [15, 16].

The effect of abiotic stressors on the growth of leaves of seedlings entails changes (both upward and downward) in the thickness of the adaxial and abaxial epidermises, the diameter of the vascular leaf bundles, and also the decrease in the diameter and size of stomata [25–28].

Anatomical and morphological changes that allow plants to withstand stressful effects are mainly focused on maintaining water use efficiency and ensuring optimal carbon metabolism for plant resistance to drought. Therefore, a change in the anatomical characteristics of a leaf under drought conditions can be considered as a significant manifestation of the regulation of photosynthesis at the morphological level.

Accordingly, the degree of change can be used as a criterion of adaptability, which can be guided by the choice of forms for the expansion of work on the introduction and hybridization in arid climatic conditions. Thus, for the survival and growth of a plant under drought stress, the degree of survival and growth of leaves is extremely

important. If leaf growth under stress conditions occurs normally, then the process of photosynthesis does not stop, and the plants are able to produce seed progeny, even if they significantly reduce yields relative to optimal conditions. However, if the leaves that function at the time of the onset of stressful conditions die off faster than new ones develop, the plant may die. At the same time, stress-tolerant forms surpass stress-susceptible in a number of quantitative indicators: the area, the mass of leaves, and the total content in them of structural units of the photosynthetic apparatus of different orders. According to the degree of change of these parameters in stressful conditions, the leaf apparatus shows its reaction to the action of negative factors.

Tolerant forms in wheat are also characterized by higher mobility in changing the typical composition of the cell population of the leaf mesophyll and, respectively, the shape of assimilating cells depending on external conditions during the leaf growth period [29]. The summary of the main directions on the functional significance of the leaf surface is that they can be grouped into two blocks: maintaining the structural and functional status of the leaf and optimizing the energy supply of photosynthesis [30].

Water deficiency contributes to a faster differentiation of tissues, with a slower overall growth, which leads to the development of xeromorphism [31]. It is believed that with a larger leaf surface, evaporation of water increases, and the resistance of plants to its deficiency decreases [32]. According to Kumakov [33], the fluctuations in yield over the years are mainly due to the enormous variabilities in leaf surface and photosynthetic potentials. A smaller volume of plant cells allows them to more easily withstand the stress that occurs when cells are squeezed during dehydration [34]. Genkel [35] indicated that in the case when xeromorphism develops under the influence of water deficiency, it correlates with drought resistance.

At the same time, a different reaction of the morphometric characteristics of the internal structure of the leaf to osmotic stress is shown. Under conditions of lack of moisture against the background of a general decrease in the size of cells, an increase in the thickness of the vascular tissue and cell wall can be observed [36]. Researchers have observed such changes as a significant decrease in leaf thickness, an increase in cuticle thickness, both an increase and decrease in xylem diameter, a reduction in phloem diameter and size of vascular bundles, and a decrease in leaf thickness and total area of the leaf mesophyll, size of stomata, and epidermis thickness for different wheat varieties, compared with the control [26, 37].

The reduction of the leaf blade area during drought is usually accompanied by an increase in the mesophyll layers and the development of palisade tissue, which allows the plant to more effectively resist dehydration [38]. Palisade parenchyma is the most high-performance type of tissue and makes the main contribution to leaf photosynthesis [39]. Developing on both sides of the leaf, it contributes to providing the plant with plastic substances while reducing the area of the photosynthesizing surface, in some way compensating for the small leaf surface of plants, which have an isolateral type of mesophyll.

Many mesophyll cells of wheat leaf plates are characterized by strong branching in cell walls, which is a necessary condition for maintaining the optimal ratio of cell surface to its volume as well as the presence of a significant proportion of intercellular spaces during evolution [40]. It was noted that during dry periods in some forms, an increase in the leaf mesophyll thickness may occur as a manifestation of the protective mechanism. In addition, it occurs due to an increase in the volume of the air cavities [41]. In plants with a loose mesophyll, an adequate supply of oxygen and carbon dioxide may be accumulated in the intercellular space, which is necessary for photosynthesis and respiration. The development of sclerenchyma around conductive bundles and the presence of columnar tissue are also one sign of the xeromorphic structure of the leaf. It is assumed that the features of growth and the lanceolate form of a plate of a leaf of wheat, where the layers of the mesophyll are

closely interconnected with conducting beams, significantly limit the diffusion of carbon dioxide to the chloroplasts of mesophyll cells [42].

It was discovered that *T. aestivum* contains 73–74% of chlorophyll-carrying cells in the lower tiers, while in the flag leaf, their share is lower and amounts to only 59% [43]. The evidence that the leaf blades of different tiers are of different quality is the fact that in the leaves of the upper phytomeres of the shoot, a certain unification of the mesophyll cells takes place and the proportion of cells with a pronounced cellular form significantly increases [40]. Such an organization of the leaf mesophyll can, in particular, contribute to the expansion of the adaptive potential of a particular species or variety to the emerging agroclimatic conditions. In general, under conditions of growing season that are different from optimal, those forms whose conditions of origin and natural habitat are characterized by a similar temperature regime and amount of precipitation with growing conditions at the moment are advantageous. This may be due to the presence of genetically fixed features that are adaptive in the geographic and climatic zone of their origin [8].

It is well known that the economic loss of the crop is greatest if the plant was subjected to stress in the juvenile stage [44]. Lack of precipitation and rapid drying of the soil can cause a quick death of a young plant. Therefore, great importance in studies of stress tolerance of agricultural crops is attached to the study of changes in the morphology and anatomy of both the root system and the leaf apparatus of wheat seedlings. It is also extremely important to study the reaction of the photosynthetic apparatus of different types of wheat to the osmotic and salt stress effects at the early stages of development. Experiments on seedlings for many decades have been an effective model system for studying a variety of physiological processes in plants, but as far as we know, there are few reports about the effects of different level water stresses on photosynthetic and metabolites activity of wheat seedlings [45].

Literature data show that during drought, both the length and width of the flag leaf and other plant morphological parameters for all wheat genotypes are significantly reduced, which, of course, affects the grain productivity. In a number of studies, a close positive correlation has been established between the size ratio of flag and subflag leaf sizes and the economic drought tolerance of varieties [46]. However, knowledge is limited on how effectively leaves of the top tier leaves, flag leaf in particular, can function under stress conditions or what adaptations could allow such functioning [25].

Callus cultures now are a convenient model object for the study of physiological processes occurring in plant cells under the influence of stress factors. Both in vivo and in vitro, cells in the light, synthesizing chlorophyll, can also acquire the ability to photosynthetically absorb carbon, that is, to implement a photoautotrophic diet typical for an intact plant.

Since embryogenesis of representatives of the Poaceae family is a photo-morphophysiological process, figuring out how stressful conditions affect the process of photomorphogenesis in vitro is of practical importance in terms of developing new test methods for assessing stress tolerance in the laboratory, and fundamental is the identification of cytophysiological conditions for the formation of photoautotrophic embryonic calluses, totipotent cells which are able to develop in various ways of morphogenesis in vitro. In the literature there are also several studies on the physiological state of photosynthetic potato and wheat cells cultivated in vitro [47, 48]. In this regard, the study of the formation of in vitro photosynthetic function is necessary for understanding the nature of the reactions of the photosynthetic apparatus of isolated tissues to stressors. Knowledge of these mechanisms will allow us to more fully characterize the relationship of photosynthetic parameters in vivo and in vitro.

This work is part of the comprehensive studies conducted 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.

Therefore, the study is certainly relevant.

Research objectives:

1. Analysis of morphophysiological and photosynthetic parameters of different wheat species under drought conditions.
2. Cytological analysis of the first leaf blades of various wheat species under drought conditions.
3. Analysis of photosynthetic parameters of the first leaves of different wheat species under drought conditions.
4. Morphophysiological and cytological analysis of the flag leaves of different wheat species under drought conditions.
5. Analysis of the influence of drought on the photomorphophysiological parameters of different wheat species in vitro.

2. Materials and methods

Six species of wheat, which differ in genomic composition and origin, from the Institute of Plant Biology and Biotechnology collection, were studied: *T. monococcum* L. (A^u), *T. dicoccum* Shuebl. (A^uB), *T. polonicum* L. (A^uB), *T. aethiopicum* Jakubz. (A^uB), *T. compactum* Host. (A^uBD), and *T. aestivum* L. (A^uBD).

In the laboratory evaluation of 10-day-old seedlings, Kozhushko's [49] methods were used as a basis. Stress conditions for seedlings were created by them exposing to 17.6% sucrose solution, visual differences in samples in growth and biomass accumulation. Control seedlings were grown in water. The experiments were carried out at a temperature of $26 \pm 2^\circ\text{C}$ and illumination of 3000 lux.

The plants which are grown in the field to the tillering stage out have been transferred to vegetative vessels of 5 liters per each of 5 plants in three replications and at regular watering were grown up to the boot stage begins. Further, a part of plants was exposed to an artificial wilting by the termination of watering during formation of a flag leaf within 7 days. The other part continued to be grown in the conditions of optimum water supply—60% of full moisture capacity. To assess the photosynthetic activity (FA) of the flag leaf on the third day, the fluorescence indices of the leaves were recorded in spring planting plants. After 5 days, the fluorescence indices of the third, fourth, fifth, and sixth leaves were registered in plants in winter planting plants. The first two leaves by this point have lost the ability to photosynthesize. On the seventh day, linear measurements of the length and area of flag and subflag leaves were carried out. By a weight method, the length and the area of flag and subflag leaves in control and stressful conditions were measured.

Conservation of plants was carried out by the method of Strasburger-Flemming: fixation was performed in 70% ethyl alcohol, and 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 [50], Permyakova [51], and Barykina [52]. 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 V400/1.3 M (Austria).

The total water content of the leaf blades was calculated from the below formula.

$TWC = (a - b) / b$; where a is the initial mass of leaves (mg) and b is the leaf mass after drying at 105°C (mg) [49].

The specific surface density of the leaf (SSDL) was calculated as the ratio of the wet weight to the unit area of the sheet.

Chlorophyll was extracted in 96% ethanol using purified glass sand to homogenize the samples. After centrifugation at 4°C (at 14,000 rpm), the chlorophyll a and b concentrations were determined with a spectrophotometer at 665 and 649 nm, respectively, with a Genesis 10 UV Scanning (ThermoScientific, USA). The concentrations were calculated according to Lichtenthaler [53].

To determine the photosynthetic CO_2/H_2O gas exchange, the sample of the sheet fragment was placed in a room temperature-cured sheet cell described earlier by Parnik [54], illuminated by a fiber-optic light guide from the illuminator (KL 1500 LCD, Shott, Germany) with halogen lamp (150 Bt, Philips, Netherlands) with the density of the light flux of PAR in the field of the object 2000 $\mu E/(cm^2 \cdot s)$. The stationary CO_2 gas exchange of the sheet was measured with an infrared gas analyzer (LI-820, LiCor, USA) in an open single-channel scheme. Transpiration was determined by the Lysk principle [55].

For the production of callus culture and plant regeneration, the technique of Gaponenko et al. [56], developed for wheat and barley, was used. The calluses of each studied wheat species were transplanted to the proliferation media of calluses (MC with addition of 2,4-D at a concentration of 1 mg/L) in 20–25 days, optimal (pH 5.6–5.7). To test the method of obtaining an actively photosynthesizing callus culture, calluses on the third passage were placed on media with a concentration of 2,4-D reduced to 1 mg/L in three variants of sucrose concentration (30, 20 and 10 mg/L) and exposed to light in the conditions of the light-cultural room, where the temperature is 25°C and the humidity is 75–80%. Some calluses continued to be cultivated in the dark. After culturing under these conditions for 20 days, the calluses were weighed and subjected to a cytological analysis. The addition of stressors (polyethylene glycol—16% w/v) simulated drought. Callus, cultured on a medium without a stressor, served as a control. In Petri dishes, 6–12 calluses were planted for each variant of the medium.

Cytological studies of callus tissues were carried out on pressed temporary preparations. The material was fixed in a freshly prepared Clark reagent (3 parts 96% ethyl alcohol: 1 part glacial acetic acid), where it was stored for 12–24 h. All leaf blades were examined microscopically at 10× magnification (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 the ×10 and ×40 lens.

The data of the experiment were analyzed statistically using Udolskaya's method [57]. Samples for analysis were means of three samples for each treatment.

3. Experimental data generalization and analysis

3.1 Analysis of morphophysiological and photosynthetic parameters of different wheat species under drought conditions

Examination of the impact of drought stress on the growth characteristics of seedlings of six different wheat species revealed significant species-specific differences in the reduction of growth of the first leaf and roots [58] (**Table 1**).

It was shown that induced drought suppressed the growth of the first leaf more than root growth of the six different wheat species seedlings. These results

demonstrate the important role of the actively functioning root system of wheat under stressful conditions [58].

The relationship of root/leaf linear sizes (an important indicator of stress) of different wheat species also increased under drought conditions. *T. polonicum*, *T. aestivum*, and *T. compactum* had the greatest increase in this ratio. The root/leaf relationship for *T. dicoccum* and *T. aethiopicum* remained virtually unchanged under stress.

The decrease in the surface area of the leaves was a response to drought (Table 2). Maximum values under drought were noted in *T. dicoccum* (83%) and *T. compactum* (86%) species.

The tendency to decrease the water content of the leaves under stresses confirmed the results of determining the relative water content (Table 2). The largest content of water with respect to control in leaves under arid conditions was characterized by *T. compactum* (88%), *T. dicoccum* (76%), and *T. aethiopicum* (71%).

3.2 Cytological analysis of the first leaf blades of various wheat species under drought conditions

The distribution of trichomes of adaxial and abaxial epidermis and the effect of osmotic stress on the change in their length were studied on the outer surface of a sheet of 10-day-old seedlings of different wheat species [10]. An increase in

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

Note: * and ** indicate significant differences at $p \leq 0.05$ and $p \leq 0.01$, respectively. Means ± standard deviations are presented.

Table 1.
Relative growth of the first leaf and roots of seedlings of different wheat species under drought conditions (17.6% sucrose, 72 h).

Species	Area, cm ²			Relative water cut, %		
	Control	Drought	% to control	Control	Drought	% to control
<i>T. monococcum</i>	3.03 ± 0.45	2.03 ± 0.30	67	1.04 ± 0.12	0.50 ± .08**	48
<i>T. dicoccum</i>	3.48 ± 0.52	2.90 ± 0.44	83	1.73 ± 0.23	1.32 ± 0.20	76
<i>T. polonicum</i>	3.56 ± 0.53	2.61 ± 0.39	73	1.83 ± 0.28	1.29 ± 0.15	71
<i>T. aethiopicum</i>	4.30 ± 0.65	3.28 ± 0.49	76	2.73 ± 0.43	2.06 ± 0.30	75
<i>T. compactum</i>	3.31 ± 0.50	2.85 ± 0.43	86	1.40 ± 0.18	1.23 ± 0.15	88
<i>T. aestivum</i>	4.35 ± 0.65	3.01 ± 0.45	69	1.64 ± 0.28	0.92 ± 0.12*	56

Note: plus/minus sign in the tables shows the relative error of the mean value; * and ** indicate significant differences at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Table 2.
The area of the first leaf of seedlings of different wheat species under drought conditions (17.6% sucrose solution, 72 h).

the length of the trichomes with increasing ploidy wheat was noted; the longest trichomes were noted for hexaploid *T. aestivum* and the shortest for diploid type of *T. monococcum* (**Figure 1**). The largest number of trichomes per 1 mm² as on the adaxial and abaxial epidermis with an average of characteristic values in the absence of stress was observed in the species *T. monococcum* (44.9 ± 5.7 and 81.8 ± 3.7 , respectively), *T. dicoccum* (53.0 ± 5.5 and 67.6 ± 4.8) and *T. aestivum* (42.0 ± 3.5 and 61.5 ± 4.0). At the same, all wheat species had greater hairiness of abaxial epidermis, except for *T. aethiopicum* and *T. compactum*, in which the hairiness of the upper and lower surfaces of the leaf did not differ.

There is a marked change in leaf pubescence seedlings of all species under drought conditions; this is consistent with literature data [47]. Under drought conditions for species such as *T. aethiopicum*, *T. compactum* and *T. aestivum*, there was noted tendency to increase the length of the trichomes of adaxial leaf surface (for species *T. compactum*, and *T. aestivum* difference was statistically significant), whereas for the species *T. aethiopicum*, *T. compactum* increased the length of the trichomes of both surfaces of the leaf. For species *T. monococcum*, there was a statistically significant reduction in the length of the trichomes of adaxial epidermis.

It was noted that an increase in ploidy increases the length of the stomata from 39.0 μm at *T. monococcum* to 72.4 μm at *T. aestivum*. Especially there was a clear dependence for the adaxial surface of leaf (**Figure 2**).

It was shown that the number of stomata per 1 mm² axial leaf epidermis of all the species is bigger than the abaxial surface (from 40.6 ± 3.9 to 90.9 ± 7.0 and from 30.2 ± 2.9 to 62.7 ± 4.3 , respectively). There was a downward trend in the number of stomata per 1 mm² of leaf surface with increasing ploidy wheat. An exception is the hexaploid *T. aestivum*; its adaxial surface in the number of stomata per unit area

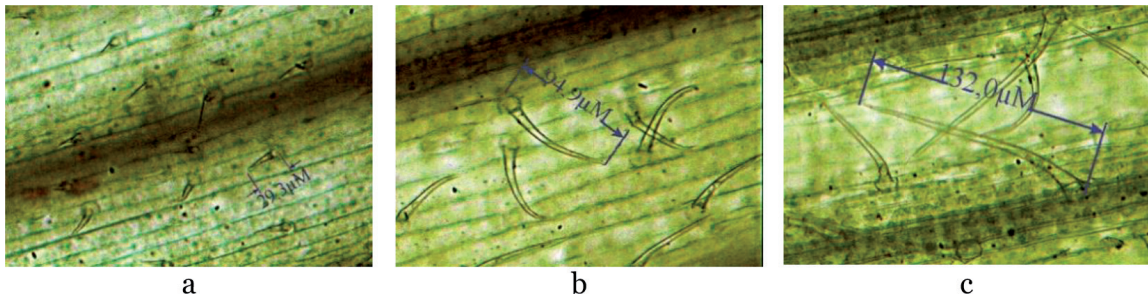


Figure 1.
The differences in trichome length depending on the ploidy of wheat species, magnification 10×.
(a) *T. monococcum* (*A*^u); (b) *T. aethiopicum* (*A*^u*B*); and (c) *T. aestivum* (*A*^u*BD*).

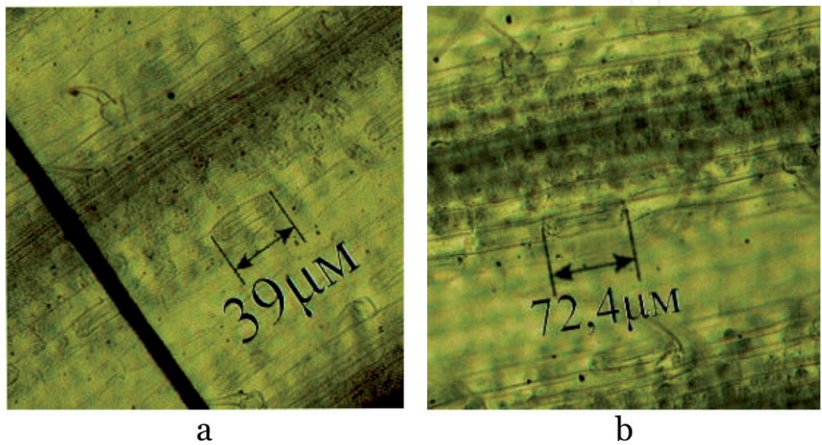


Figure 2.
The differences in stomata length depending on the ploidy of wheat species, magnification 10×.
(a) *T. monococcum* (*A*^u) and (b) *T. aestivum* (*A*^u*BD*).

exceeds the tetraploid species. The total area of stomata was: for adaxial surface, from 5 to 12%; for abaxial, from 3 to 5% of leaf area (1 mm²); and for *T. aestivum*, it was also maximal [10].

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 and “mucilaginated” for less stable forms, which causes a slight increase in the linear values of the width. In drought conditions there was a significant decrease in the parameters of length and width of stomata adaxial epidermis of species *T. aestivum*.

It was noted for the species *T. dicoccum* and *T. aethiopicum* the decrease in the magnitude of the specific density of leaf surface SDLS in stressful conditions was the most significant (Figure 3). Probably, the mesophyll of the first leaf of these species was packed less tightly in drought conditions than in control.

For a detailed understanding of the mechanisms of changing leaf density in osmotic stress, anatomical studies of the internal structure of leaf blades of 10-day seedlings of the studied wheat species were carried out.

This species was differenced in a number of anatomical parameters (Table 3).

From the presented data, it follows that the greatest thickening of both the abaxial and adaxial epidermis under stress conditions is characteristic of the species *T. dicoccum*, *T. polonicum*, and *T. aethiopicum*. A similar arrangement of mesophyll cells allows us to characterize the type of structure of leaf mesophyll in all studied wheat species as a loose cellular-isolateral-palisade. It is characteristic for the *T. aethiopicum* and *T. aestivum* under drought conditions (thickening of mesophyll was 115.3% and 117.4%, respectively, to control). In this experiment drought stress causes thickening of the central veins of almost all studied species except for wheat *T. aestivum*. As follows from the presented data, in most of the species except for *T. monococcum*, *T. aestivum* and size of the central vascular bundle during drought stress was increased, which is an indicative of its high adaptation ability to the drought. Consequently, indicators such as an increase in the stress of the sizes of protective and mechanical tissues, as well as mesophyll, can serve as criteria for selecting stress-resistant forms of wheat. By analyzing the data virtually in all considered anatomical parameters of the leaves, we can say of a higher adaptive capacity of tetraploid wheat species *T. dicoccum*, *T. polonicum* and *T. aethiopicum* than hexaploid [10].

3.3 Analysis of photosynthetic parameters of the first leaf blades of various wheat species under drought conditions

A more uniform distribution of chloroplasts in the cells of the leaf blade in the absence of stress was observed. Chloroplasts were concentrated in the areas of vascular bundles under stressful conditions (Figure 4).

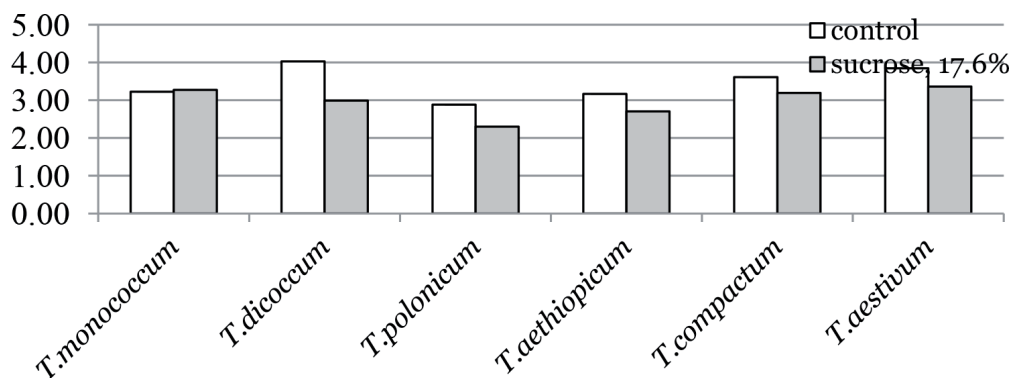


Figure 3. SSDL of leaf blades of different wheat species of in stress conditions, g/dm².

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>	38.25 ± 2.50	31.50 ± 1.98	117.48 ± 17.80	490.57 ± 0.90*	39,408.14*
<i>T. dicoccum</i>	37.25 ± 2.02	38.80 ± 2.01	158.22 ± 2.30*	480.61 ± 1.80*	32,614.23*
<i>T. polonicum</i>	32.77 ± 0.65	26.57 ± 3.31	146.89 ± 0.98*	470.62 ± 3.40*	23,235.22*
<i>T. aethiopicum</i>	39.07 ± 1.70	36.97 ± 2.10	117.34 ± 0.90*	490.57 ± 2.30*	39,408.14*
<i>T. compactum</i>	32.60 ± 3.04	34.82 ± 2.60	105.73 ± 4.60	377.47 ± 7.80	11,309.73
<i>T. aestivum</i>	36.65 ± 1.42	27.94 ± 1.40	106.18 ± 3.80	411.71 ± 2.30	33,979.45
Drought					
<i>T. monococcum</i>	37.25 ± 1.70	32.75 ± 0.50	144.29 ± 0.60	481.36 ± 0.70	28,952.92
<i>T. dicoccum</i>	42.25 ± 1.70*	40.05 ± 0.80*	145.25 ± 0.80	529.91 ± 1.90*	33,575.91*
<i>T. polonicum</i>	43.80 ± 0.80*	39.37 ± 0.70*	145.23 ± 0.50	553.59 ± 6.20*	54,739.11*
<i>T. aethiopicum</i>	41.80 ± 0.80*	39.37 ± 0.70*	135.23 ± 0.50	553.59 ± 6.20*	34,739.22*
<i>T. compactum</i>	38.98 ± 0.20	35.47 ± 0.50	138.03 ± 2.90	488.26 ± 4.50	22,167.12
<i>T. aestivum</i>	31.67 ± 2.80	28.94 ± 1.60	124.67 ± 0.70	374.99 ± 3.50	16,361.22

Note: plus/minus sign in the tables shows the relative error of the mean value; *, ** indicate significant differences at $p \leq 0.05$ and $p \leq 0.01$ respectively.

Table 3.
Morphometric parameters of the first leaves of different wheat species under control and drought stress conditions (17.6% sucrose solution, 72 h).

Earlier, it was shown that the chlorophyll content in leaf blades was species-specific in the absence of stress, but it was independent of the ploidy level of the species studied, but *T. aestivum* had the highest concentration of chlorophyll (a+b) in leaf blades under normal conditions (control). Under stressful conditions the chlorophyll content of all studied species except for *T. compactum* and *T. aethiopicum* decreased significantly (by 75–84%), but for *T. dicoccum* it was increased [45] (**Figure 5**).

The ratio of chlorophyll a/b in our experiments was stable, independent of the changes in total chlorophyll content, suggesting that the osmotic stress applied in this study did not cause significant structural changes in the photosynthetic apparatus of seedlings of different wheat species [46].

The study of photosynthetic CO₂ gas exchange, attributed to the chlorophyll (a + b) content in the leaf, revealed that the assimilation index remained practically unchanged under drought conditions only in *T. dicoccum* (**Figure 6**).

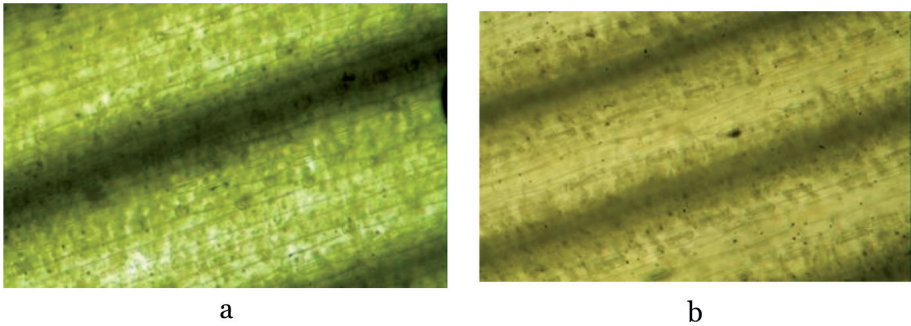


Figure 4.
Coloring of leaf blades of *T. monococcum* seedlings under (a) control and (b) drought stress conditions (17.6% sucrose solution, 72 h), magnification 10×.

Transpiration of leaves, correlated to the chlorophyll content (a + b), during drought, increased in *T. aestivum* and in *T. dicoccum* and in *T. dicoccum*—almost four times (Figure 7).

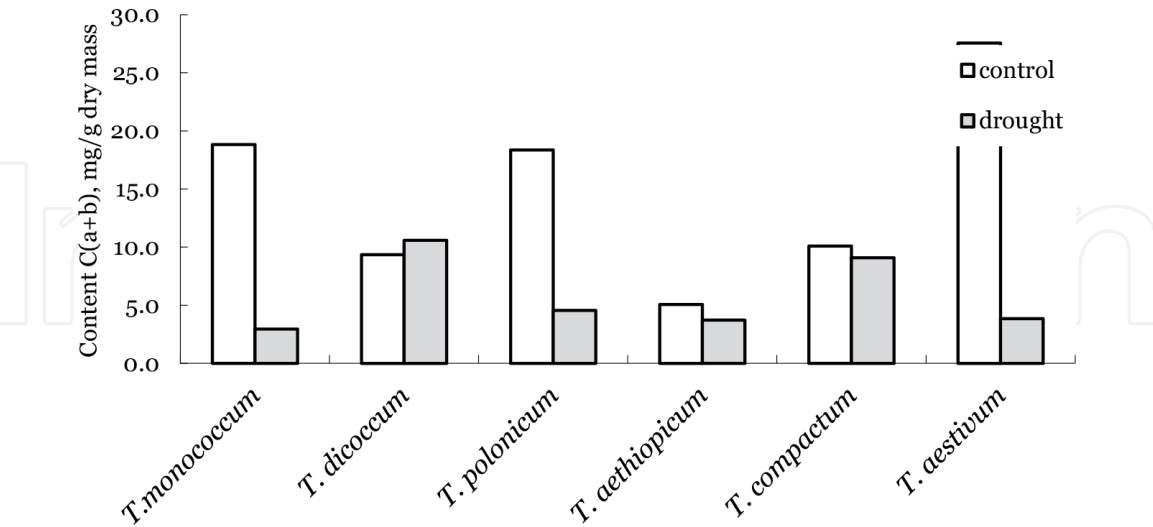


Figure 5. Concentrations of chlorophyll (a + b) under control and drought conditions (17.6% sucrose solution, 72 h). Note: Different letters above the bars represent significant differences at $p \leq 0.05$.

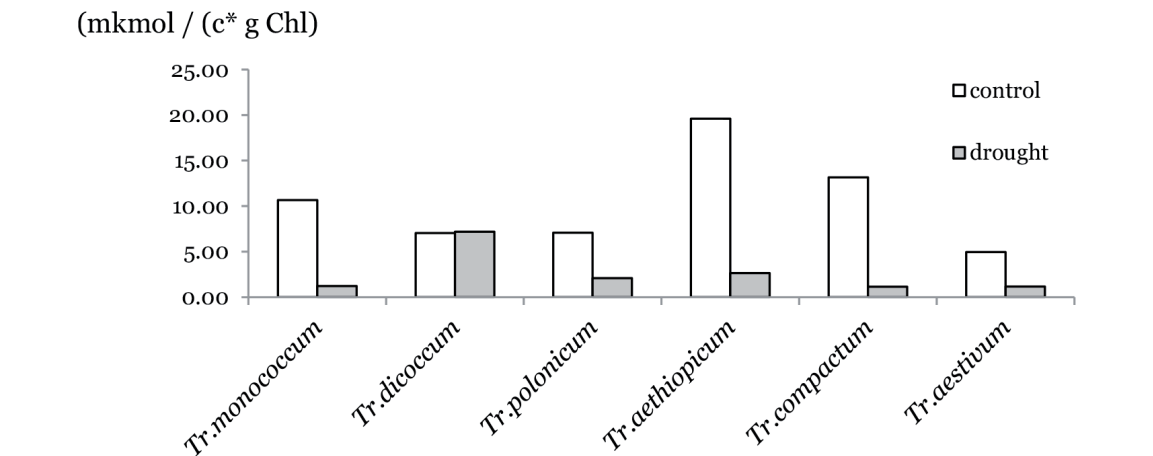


Figure 6. The level of CO₂ assimilation under control and drought conditions (17.6% sucrose solution, 72 h) (mkmol/(c* g Chl)).

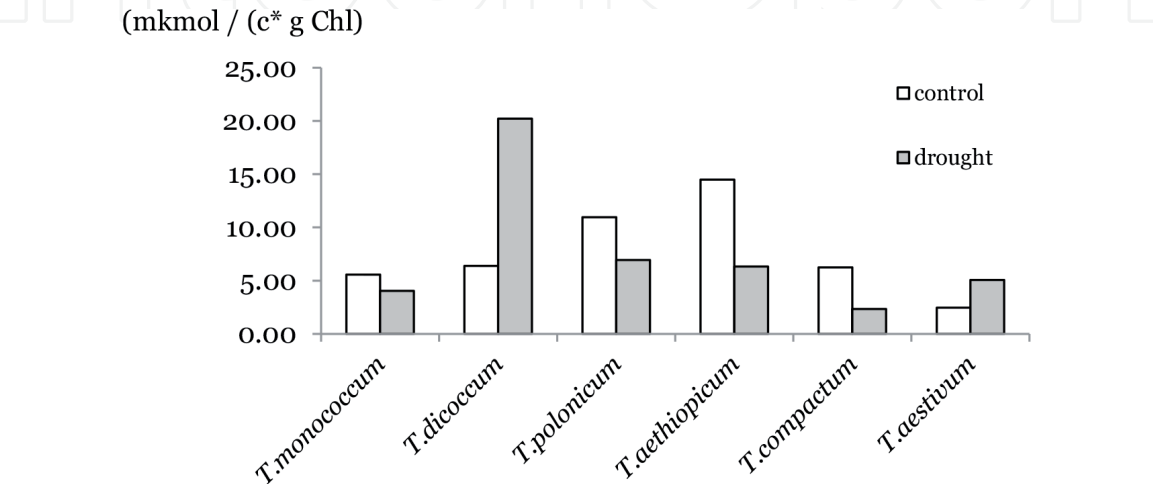


Figure 7. Intensity of transpiration of different wheat species under control and drought conditions (17.6% sucrose solution, 72 h) (mmol/c* g Chl).

These results are consistent with literature data in which the increase in transpiration intensity in conditions of water deficiency in the Saratovskaya-29 strain correlates with the specificity of response to osmotic stress, which is not typical for most plants but is one of the signs of high drought resistance of this variety [59]. Perhaps this sign is a pronounced indicator of drought resistance and for the species *T. dicoccum*.

We can also assume that one of the reasons for the increase in the rate of transpiration, the renewal of growth, and the active functioning of the leaf in the *T. aestivum* and *T. dicoccum* species under induced drought conditions was the increase in water inflow from the roots, which is provided by the active work of the little stress-damaged root system of these species.

Reduction of stomatal conductance not only reduces water losses during transpiration but also increases the efficiency of its use. Using a parameter, usually in the literature denoted as WUE (water use efficiency, ratio of intensity of assimilation of CO₂/transpiration) or TE (transpiration efficiency) [36, 60], we estimated how great the loss of water by the plant is by assimilating a unit of carbon. The results of our experiments showed a significant reduction in the vegetative WUE both under artificial drought stress conditions (Figure 8).

The change in the specific surface density of the leaf (SSDL) was evaluated. A negative relationship was found between the indices of the SSDL and the WUE. But if under control conditions, it was $r = -0.6^{**}$ and under the conditions of induced drought, it decreased by half ($r = -0.3^{*}$), which indicates the importance of taking into account the structural changes in leaf blades in connection with the efficiency of water exchange under stressful conditions.

Besides, we used the “saturation pulse” method for the detection of the effects of drought on photosynthesis of leaves different wheat species. As shown [45], most species had higher and light-dependent thermal dissipation (Y(NPQ)) under induced drought than in controls, indicating the presence of lesions in FS II. But there were no significant differences in the maximum quantum yield of PSII (Fv/Fm ratio).

3.4 Morphophysiological and cytological analysis of the flag leaf blades of various wheat species under drought conditions

It was revealed that the length of the two upper leaves of plants did not change significantly under the influence of drought and is made up by the flag sheet from 81.7% (*T. aethiopicum*) to 107.5% (*T. dicoccum*) to control (according to the subflag sheet—104.9% (*T. dicoccum*) to the control) [60]. The area of the two upper leaves

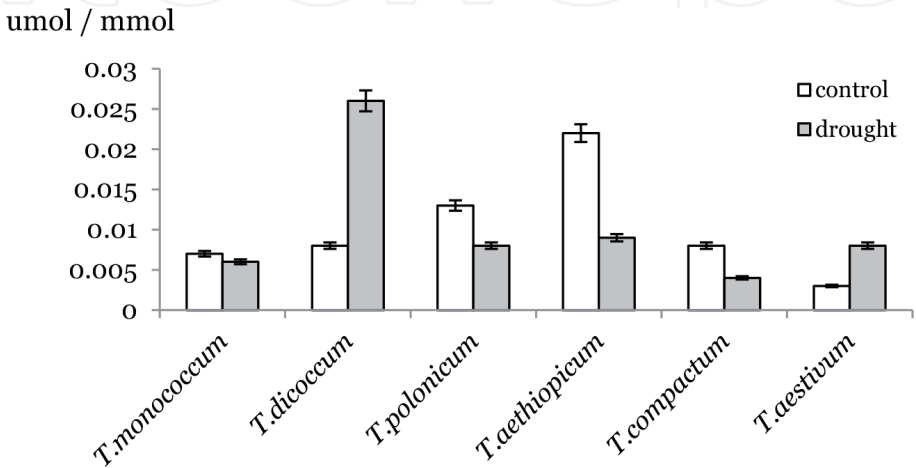


Figure 8.
Change in plant WUE of different wheat species under control and drought conditions (17.6% sucrose solution, 72 h), umol/mmol.

of the studied wheat species was more strongly affected by the growth conditions. Thus, according to the flag sheet, the area under drought conditions was from 41.3% (*T. aethiopicum*) to 79.1% (*T. aestivum*) to control; the area of the subflag leaf is from 58.9% (*T. monococcum*) to 101.2% (*T. dicoccum*) to the control (**Table 4**).

Observed increases of flag and subflag leaves area at tetra- and hexaploid species in comparison with a diploid *T. monococcum* can be a consequence of increase of total number of cells counting on a leaf, increase of the number of cellular divisions and an intensifications of division and stretching processes [33, 35].

Such species as *T. aestivum* and *T. dicoccum* were characterized by the largest area of a subflag leaf (91 and 101% to control, respectively), with some decrease for ratio of the flag/subflag area under drought from 125.5 to 109.1% in *T. aestivum* and from 86.6 to 66.2% in *T. dicoccum*, but remain quite high. Thus, change of a ratio of a flag/subflag is directly connected with preservation of functional abilities of a subflag leaf and with extent of development of a flag leaf under drought conditions. At more tolerance species decrease in this indicator was the smallest (**Table 4**).

Structural transformations of leaves observed in different phases of plant ontogeny under drought are described as changes in the direction of amplification of xeromorphism [40]. The flag leaf may be inferior in length and density of pubescence to the rest [38]. According to our data [35], in di- and tetraploid species, the upper side of the flag leaf is pubescent less than the lower side. On the upper side of the leaf, the hairs are concentrated mainly on the veins, keeping the same density throughout the entire width of the leaf blade. In this case, more hairs occur on the central vein and less—on the lateral. On the lower side of the leaf, the trichomes are placed evenly over the surface, both on the veins, and between them.

The drought that occurred during the formation of the flag leaf had a significant impact not only on the density of pubescence of the leaf blades but also on the length of trichomes of some species (**Table 5**). In the case of the diploid species, *T. monococcum*, trichome length reduction in both adaxial and abaxial epidermises was noted, while tetraploid species *T. dicoccum* and *T. aethiopicum* showed an increase in adaxial epidermal trichomes length. But in *T. aethiopicum* and *T. aestivum*, we show an increase of the abaxial trichomes length, and in the species *T. aestivum*, it is expressed in greatest extent.

It is known that the larger the stomata and the smaller the cells, the more xeromorphous the species [41]. In accordance with our data [35], the largest xeromorphism of the flag leaf is also characteristic for tetraploid wheat species. The regularity of the location of a larger number of stomata on the lower (abaxial) epidermis of the leaf blade was remained. As in our experiment on seedlings [10], the stomata size was dependent on the ploidy of wheat species. The deficiency of soil moisture did not significantly affect the length of the stomata of most of the studied forms, which indicates a significant genetic stability of this trait [61].

The importance for the vital activity of plants of the internal structure of the flag leaf, carrying out basic water and gas exchange, during critical periods from 20 days to flowering up to 10 days after flowering and during the period of grain filling, is unconditional. In the absence of a stress, the maximum values of adaxial epidermis characterized the species as *T. dicoccum* and *T. aestivum* (33.49 and 34.00 μm , respectively) regardless of their ploidy. The maximum values of abaxial epidermis were at hexaploid species of *T. compactum* (26.45 μm) [35] (**Table 6**).

The maximum thickness of a mesophile is noted at a tetraploid of *T. dicoccum* (352.89 μm). The tetraploids of *T. dicoccum* and *T. aethiopicum* had a maximum thickness of the central vein (951.60, 931.57, and 926.86 μm , respectively). And the biggest size of the central conducting bundle is noted at a diploidic species of *T. monococcum* and a hexaploid of *T. compactum* (35,155.73 and 38,424.32 μm , respectively).

Species	Flag leaf, cm ²		% to control	Subflag leaf, cm ²		% to control	Flag/subflag, %	
	Control	Drought		Control	Drought		Control	Drought
<i>T. monococcum</i>	8.2 ± 0.3	5.7 ± 0.2	70**	14.3 ± 0.6	8.5 ± 0.3	59**	57	68
<i>T. dicoccum</i>	36.2 ± 1.6	28.0 ± 1.4	77	41.8 ± 1.8	42.3 ± 1.9	101	87	66
<i>T. polonicum</i>	29.3 ± 1.2	21.4 ± 0.9	73*	30.0 ± 1.5	17.9 ± 0.8	60**	98	119
<i>T. aethiopicum</i>	47.2 ± 1.9	19.5 ± 0.8	41**	55.8 ± 2.3	35.1 ± 1.8	63**	85	56
<i>T. compactum</i>	26.3 ± 1.0	17.6 ± 0.7	67**	35.7 ± 0.4	17.4 ± 0.7	49**	74	101
<i>T. macha</i>	28.7 ± 1.1	19.0 ± 0.8	66**	31.3 ± 1.6	19.7 ± 0.9	63**	92	97
<i>T. aestivum</i>	33.5 ± 1.7	26.5 ± 1.3	79	26.7 ± 1.2	24.3 ± 1.1	91	126	109

Note: The plus/minus sign in the tables shows the relative error of the mean value. The * and ** signs show the reliability of the t-test differences at 0.05 and 0.01 significance level with respect to the control.

Table 4.
The area of a flag and subflag leafs at different species of wheat in the induced drought (the termination of watering, 7 days).

Species	The length of the trichomes, μm			
	Adaxial epidermis		Abaxial epidermis	
	Control	Drought	Control	Drought
<i>T. monococcum</i>	35.5 \pm 1.7	21.2 \pm 2.6**	44.0 \pm 4.3	21.1 \pm 3.5**
<i>T. dicoccum</i>	21.4 \pm 3.1	30.1 \pm 1.4**	32.1 \pm 3.8	32.2 \pm 2.1
<i>T. polonicum</i>	19.4 \pm 2.4	18.7 \pm 2.4	23.5 \pm 2.6	22.7 \pm 2.7
<i>T. aethiopicum</i>	54.8 \pm 0.9	59.8 \pm 2.6	65.5 \pm 1.8	44.3 \pm 4.4**
<i>T. compactum</i>	21.7 \pm 2.5	21.0 \pm 3.4	22.5 \pm 0.7	20.1 \pm 2.9
<i>T. aestivum</i>	72.8 \pm 3.4	34.2 \pm 1.5**	21.8 \pm 4.1	74.5 \pm 4.2**

Note: The plus/minus sign in the tables shows the relative error of the mean value. The *, ** signs show the reliability of the t-test differences at 0.05 and 0.01 significance level with respect to the control.

Table 5.
Change the size of trichomes of flag leaf blades of different species of wheat depending on the influence of stress factors (the termination of watering, 7 days).

Species	Width of adaxial epidermis	Width of abaxial epidermis	Thickness of a mesophile	Thickness of the central vein	The size of the central conducting bundle
Control					
<i>T. monococcum</i>	20.5 \pm 0.6	19.8 \pm 0.1	210.5 \pm 1.9	457.8 \pm 3.3	35,155.7*
<i>T. dicoccum</i>	33.5 \pm 0.4**	19.8 \pm 0.1	352.9 \pm 3.3*	951.6 \pm 7.9**	24,859.6
<i>T. polonicum</i>	29.3 \pm 1.9*	22.2 \pm 0.0*	209.2 \pm 2.6	409.4 \pm 4.8	29,054.3*
<i>T. aethiopicum</i>	27.4 \pm 0.2*	16.8 \pm 0.1	252.7 \pm 3.1	931.6 \pm 5.9**	18,594.8
<i>T. compactum</i>	27.4 \pm 0.3*	26.5 \pm 0.1**	134.8 \pm 2.2	512.9 \pm 4.9	38,424.3*
<i>T. aestivum</i>	34.0 \pm 0.9**	19.2 \pm 0.01	215.3 \pm 2.2	520.1 \pm 4.5	22,244.7
Drought (the termination of watering, 7 days)					
<i>T. monococcum</i>	30.2 \pm 0.6**	17.0 \pm 0.8	90.5 \pm 1.7	298.7 \pm 3.7	8807.1
<i>T. dicoccum</i>	28.1 \pm 0.5*	24.8 \pm 0.7*	141.9 \pm 2.3*	577.9 \pm 5.6*	19,775.1*
<i>T. polonicum</i>	28.9 \pm 0.7*	18.8 \pm 0.6	65.1 \pm 0.9	789.2 \pm 3.2**	18,612.9
<i>T. aethiopicum</i>	22.7 \pm 0.3	18.3 \pm 0.2	99.1 \pm 1.8	371.1 \pm 3.9	18,612.9
<i>T. compactum</i>	29.4 \pm 0.3*	21.5 \pm 0.1	163.5 \pm 2.1*	510.3 \pm 4.7*	18,612.9
<i>T. aestivum</i>	26.0 \pm 0.4	24.0 \pm 0.3*	183.5 \pm 2.2*	483.2 \pm 4.4*	20,991.8*

Note: plus/minus sign in the tables shows the relative error of the mean value; *, ** indicate significant differences at $p \leq 0.05$ and $p \leq 0.01$ respectively.

Table 6.
Morphometric parameters of a flag leaf of different species of wheat in control and drought conditions, μm .

In control the maximum thickness of a mesophile is noted for the tetraploid *T. dicoccum* (352.89 μm). The tetraploids *T. dicoccum* and *T. aethiopicum* had also a maximum thickness of the central vein (951.60, 931.57 and 926.86 μm , respectively).

The drought exerted various impacts on parameters of a flag leaf of the studied wheat species. In hexaploid group the average value of thickness of a mesophyll increased by 50–56% in comparison with tetra- and diploids, respectively.

On the flag leaf, we did not find such clear relations by formation of anatomical parameters with ploidy under stress as on the seedlings. This may be due to the different quality of leaf blades of different tiers. Since the conditions for the formation of the upper leaves, in contrast to the lower ones, vary to a very wide extent, respectively, the morphological characteristics of the leaves of the upper tiers have a much greater range of variation. At the hexaploid species, more “degrees of protection” to a lack of moisture than di- and tetraploid were observed. In general, the anatomical structure of the leaves of various wheat species under the influence of drought is labile and reflects the degree of adaptation of individual species.

As appears from the submitted data, in control conditions we could see the impact on formation of the area of a leaf thickness of adaxial (top) epidermis, thickness of the central vein ($r = 0.7^*$), and indirectly influenced thickness of a mesophyll ($r = 0.4$) (**Figure 9**).

But the drought that completely leveled the importance of thickness of the top epidermis has reduced the importance of a mesophyll ($r = 0.3$) and the central vein ($r = 0.5^*$) but has brought to the forefront influence of the sizes of the central conducting bundle ($r = 0.9^*$) and thickness of abaxial (lower) epidermis ($r = 0.6^*$).

As the lower epidermis of a leaf is not covered with a cuticle and it has bigger quantity of stomas than the top, what making it the main location of gas exchange and transpiration, its thickening at drought is an important adaptation sign.

The decrease in the sizes of the main conducting bundle at a drought is directly connected with reduction of the sizes of area of a xylem which is directly responsible for the ability of plants to absorb water and to carry out nutrients, changing the diameter of a vessel. Consequently, such indicators as thickness of abaxial (lower) epidermis and the size of the central conducting bundle are correlatively connected with growth indicators of a flag leaf in case of a drought and do not depend on the ploidy of the studied species. Thus, it is eaten out of the maximal capability to adaptation to drought conditions in the late stages of ontogeny of a flag leaf of *T. dicoccum* and *T. aestivum* wheat species.

3.5 Analysis of the influence of drought on the photomorphophysiological parameters of different wheat species in vitro

Embryogenic photomorphogenic calluses for all studied types of wheat were obtained. The lowest percentage of callusogenesis from immature embryos was noted in the diploid species *T. monococcum* (14.6%), and hexaploid forms were characterized by the highest frequency of callusogenesis (87–91%).

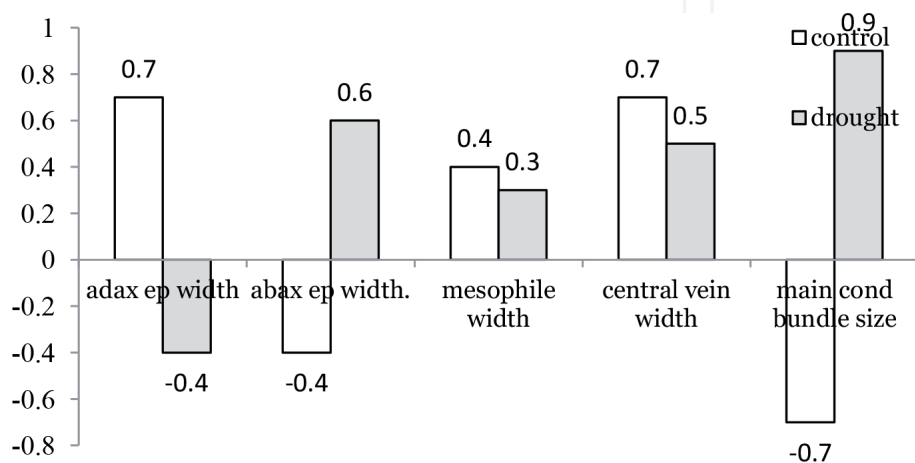


Figure 9.
Influence of drought on correlative interactions between the leaf area and basic anatomic parameters of a flag leaf.

It was revealed that the growth rates of callus tissue in the studied species and hybrids of wheat in the dark and in the light differed on media differing in the level of carbohydrate nutrition. At 20 g/L, the accumulation of biomass, as a rule, was the greatest (83–117%) [62, 63].

A comparative analysis of the morphophysiological characteristics of calluses, which was cultivated in the dark and in the light, showed that the calli growing in the dark had a disordered loose structure, callus tissue watered, easily disintegrating into individual cells.

At 20% sucrose concentration in the nutrient medium, we pronounced zones of formation of morphogenic structures in calluses were revealed. They lacked almost no chlorophyll in the absence of lighting, but when the callus was exposed, they were green. The isodiametric shape of the cells, the intensely colored cytoplasm, and the tight adherence of the cells to each other made it possible to characterize the cells of such callus as meristematically active, with photomorphogenic light, which also agrees with the literature data [53]. In the light, in the greening meristematic zones, chlorophyll-containing areas (CCA) of formation of tracheid structures and conducting beams are clearly marked, which indicates the onset of the regeneration process (**Figure 10**). These processes were characteristic of the calluses of all the species studied. Thus, it is possible to draw a positive conclusion about the possibility of obtaining strains of the photoheterotrophic callus culture from heterotrophic calli of various wheat species with optimum light conditions and carbohydrate composition of the nutrient medium.

It was shown that the onset of the formation of chlorophyll in callus indicates the onset of morphogenesis. Optimum conditions for intensive formation of green

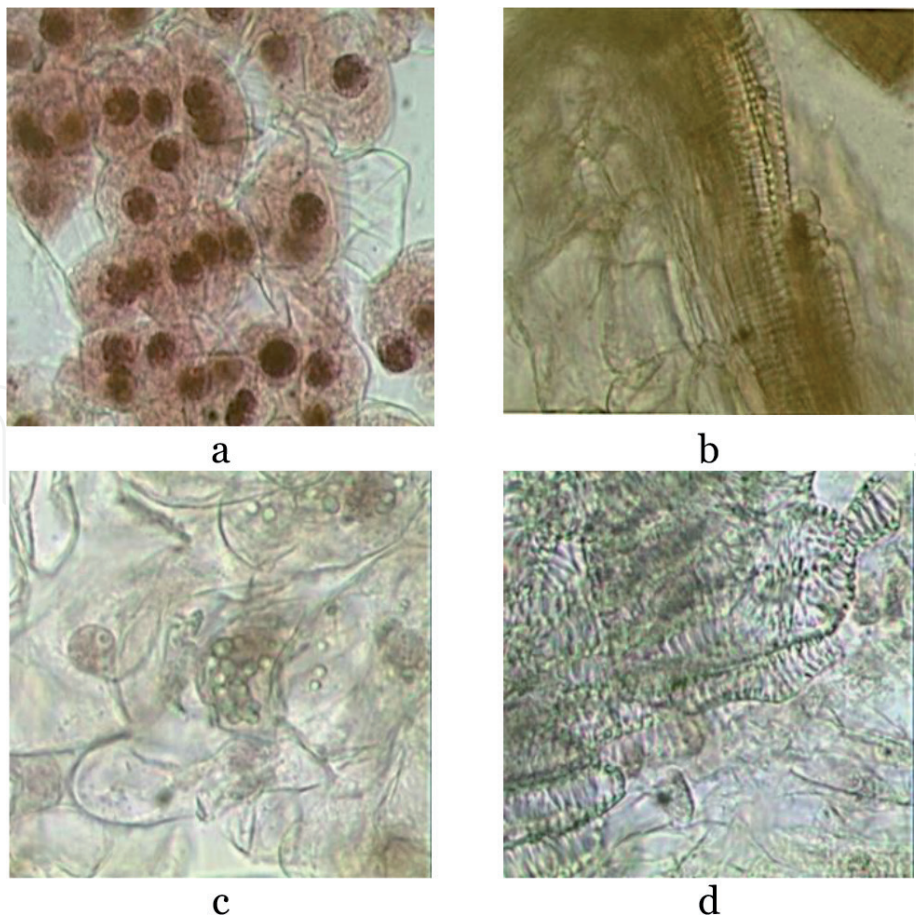


Figure 10. Cells of calli growing at 20% sucrose concentration, magnification 40×. (a) meristematic cells of morphogenic callus growing in the dark; (b) site of formation of tracheid structures; (c) chloroplasts in callus cells in the light; and (d) green zone of regeneration start.

pigments in cells were 20 g/L sucrose in a nutrient medium and stimulation of callus tissues by illumination. Most of the calluses had a high regenerative capacity during further cultivation for 2–3 weeks. At the same time, the correlation between the accumulation of callus biomass in the studied wheat species and their regenerative capacity under optimized conditions was quite high ($-r = 0.9^{***}$) (**Figure 11**).

Reliable correlation between the average size of the main spike of the studied wheat species in the field (averaged perennial data) and the ability to accumulate biomass by calluses of these species in vitro was $r = 1^{***}$ (**Figure 12a**), and correlation between the main spike and ability of callus tissues to regenerate a plants $r = 0.7^{**}$ was revealed (**Figure 12b**).

Thus, these results show that the optimization of the conditions for the cultivation of calluses of various types of wheat has been achieved both for obtaining photomorphogenic callus culture and plant regeneration and for obtaining callus tissue of sufficient biomass for various experiments, including resistance to abiotic stresses in vitro. According to the results of this research, the Patent of the Republic of Kazakhstan No. 1642 for the utility model “Method for obtaining of photosynthesizing callus culture of wheat” in 2016 was obtained.

The analysis of drought effect on the callus tissues of different wheat species showed that calluses of tetraploid species of *T. dicoccum*, *T. polonicum*, *T. aestiopicum*, and hexaploid *T. aestivum* in control conditions were significantly superior to other species in terms of the growth of raw biomass. In drought conditions in tetraploid wheat forms, biomass depression was also less pronounced than in others (**Figure 13**).

We showed that photomorphogenic calluses even in stressful conditions formed several greening loci (**Figure 14a**). Chloroplasts in zones of somatic embryogenesis were similar to chloroplasts of leaf mesophyll cells. But in contrast to intact plants, in which chloroplasts are usually located near the cell walls, in the callus cells, the arrangement of chloroplasts is chaotic (**Figure 14b**).

The most active processes of photomorphogenesis under stress conditions are found in callus developing in the light, which have a relatively small increase in the biomass of cell colonies. This reflects the negative genetic mechanism of interaction between growth processes and photosynthesis in the formation of plant tissue in vitro and is consistent with the literature data [64].

Thus, a large percentage of regenerating plants under conditions of induced drought in vitro was noted in species *T. dicoccum* (67%) and *T. aestivum* (50%). With prolonged exposure to stress, plasmolysis observed in most of the studied forms and the proportion of vacuolated cells that lie in the lower part of the callus and contact with the nutrient medium was decreased. The increase in the duration

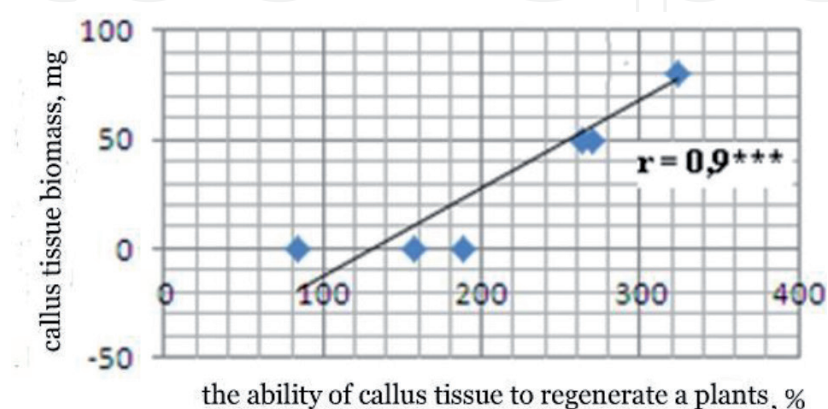


Figure 11.
The correlations between the accumulation of biomass and the regenerative capacity of callus tissues of various wheat species in vitro.

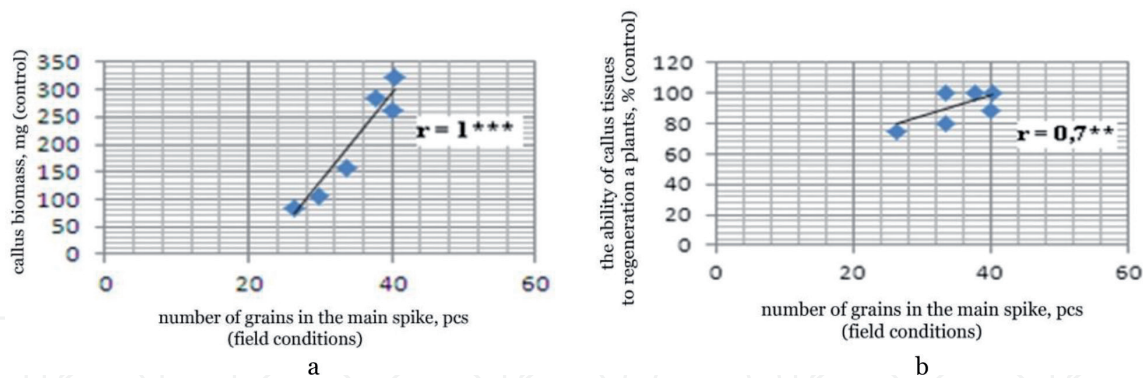


Figure 12. Correlation links between (a) regenerative ability of callus tissues and (b) biomass of calluses of different wheat species and number of grains in the main spike (average long-term data).

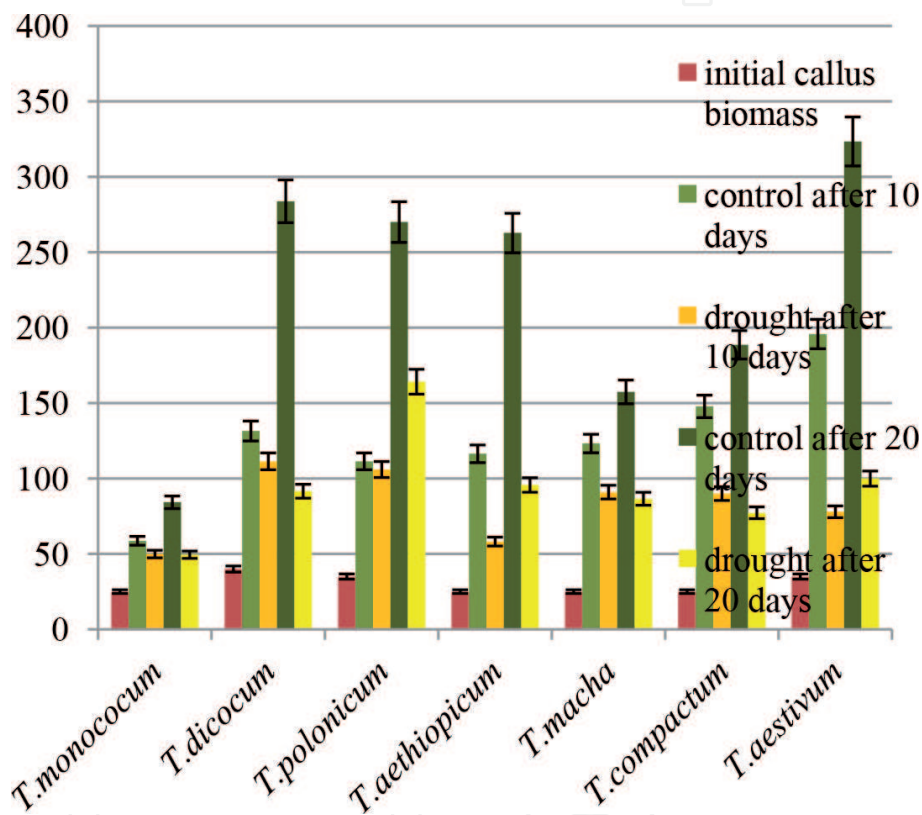


Figure 13. Growth of biomass of calli of various wheat species in vitro in control and induced drought (PEG-6000, 16% (weight/volume)) conditions.

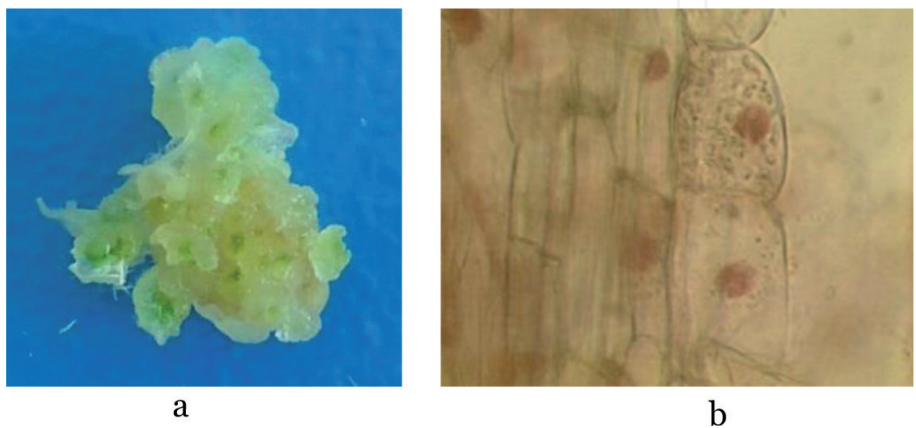


Figure 14. Regeneration processes (hemogenesis) in callus tissue of wheat, magnification 40×. (a) Numerous regeneration zones in callus tissue and (b) chloroplasts in callus cells.

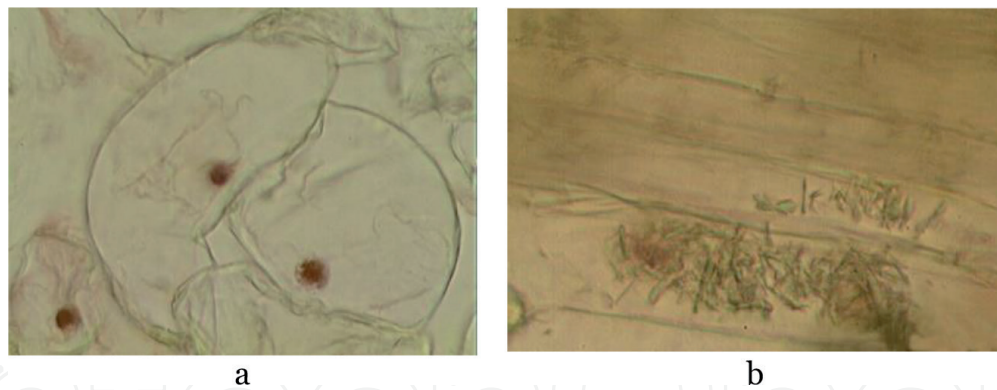


Figure 15. Processes of degeneration of photomorphogenic callus tissue of wheat under conditions of induced drought, magnification 40×. (a) Plasmolysis in large vacuolated cells of *T. compactum* and (b) destruction of CCA in callus cells of *T. polonicum*.

of the stressful action led to the processes of degeneration of the photomorphogenic tissues and the destruction of CCA and trachea-like structures, primarily in the calluses of less stable species (**Figure 15**).

The destruction of synthesized pigments and the cessation of their synthesis *de novo* indicate a stress-induced disturbance of the mechanisms coordinating the formation of pigments and their protein carriers. In general, the proportion of callus cells containing chloroplasts, as well as the osmotic characteristics of callus cells, can serve as indicators of the photomorphogenetic competence of the species under stress conditions *in vitro*. The *T. dicoccum* and *T. aestivum* species, in which the formation of CCA under stress conditions prevailed over the processes of rhizogenesis, can be considered the most resistant to drought stress *in vitro*.

4. Conclusion

The present study, which involved wheat species with different genome contents, includes a complex analysis of photosynthetic apparatus resistance to drought stress *in vivo* at different levels of organization as well as *in vitro* on callus cultures, which was conducted for the first time.

Six species of wheat were studied: *T. monococcum* 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).

The new results were obtained. These experiments revealed a number of common nonspecific mechanisms regulating growth and photosynthetic activity of the leaf apparatus of seedlings of different wheat species seedlings under drought stress. Furthermore, species-specific differences in the response to induced drought were demonstrated. Drought stress had effect on the leaf apparatus of juvenile plants. Among the wheat species with different origins, levels of ploidy and genomic composition, a variety of evolutionary mechanisms for protection against exposure to stressors in the form of tolerance, were identified in tetraploid species and especially *T. dicoccum*.

T. dicoccum as well as *T. aestivum* species also had the largest under flag leaf area at late stages of ontogeny. They also had in comparison with other studied species the maximum flag leaf area under drought conditions compared to optimal ones. The changes in flag/subflag ratio appeared to be closely connected with subflag functionality retention as well as with flag development level under stress conditions. The more tolerant forms showed less decrease of this ratio under drought.

Changes in growth activity of different wheat species appeared to be connected changes in photosynthetic apparatus work under stress conditions. Also different mechanisms developed with evolution can be involved in resistant processes against abiotic factors influence.

The study of photosynthetic CO₂ gas exchange at the background of significant decrease of assimilation and transpiration of most studied species showed high levels of these parameters in *T. dicoccum* as well as *T. aestivum* samples. The significant decrease of plant WUE was described under drought stress. A negative relationship was found between the indices of the SSDL and the WUE, which decreased under stress conditions, which indicates the importance of taking into account the structural changes in leaf blades in connection with the efficiency of water exchange under stressful conditions.

The anatomic features of inner and external surfaces of leaves of all studied wheat species growing under optimal and stress conditions were described. Most of studied anatomic parameters of seedlings testified higher adaptation ability of tetraploid wheat species compared to hexaploid species. The enlargement of resistant and mechanical tissues and mesophyll size appeared to be a good criteria of stress-tolerant form selection at early growth stages.

Leaf blades of hexaploid species have more adaptive mechanisms than leaf blades of di- and tetraploid species at late growth stages. It was revealed that abaxial epidermis thickness and central conducting bundle size correlate with flag size under drought conditions. So these parameters can become suitable criteria of selection of wheat drought-tolerant forms. *T. dicoccum* as well as *T. aestivum* had the highest adaptive potential to drought according to data on the anatomy of leaves of upper tiers.

The maximum in vitro callusogenesis frequency was detected in immature embryo culture of hexaploid species, the minimum of diploid species *T. monococcum*. The tetraploid species calluses had higher weight than calluses of other species. The species specific of CCA formation was shown. It was revealed that callusogenesis, morphogenesis, and CCA formation parameters were lower in mature embryo culture than in culture, developed from immature embryos.

Optimization of different wheat species callus culture cultivation was reached for the purpose of photomorphogenic callus culture production. Significant correlation between studied species callus weight and regeneration capacity was found out. Correlations between crop yield and growth parameters of callus culture of those species under optimized conditions of in vitro cultivation were shown.

The results of the present project may be applied in plant physiology, genetics, biotechnology, and plant breeding. The developed approaches may be used for screening of wheat selection collection for drought-tolerant forms. These methods provide and sustain morphophysiological, anatomic, physiological, and biotechnological markers at different stages of ontogenesis in callus cultures in vitro.

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Optimization of different wheat species callus culture cultivation was reached for the purpose of photomorphogenic callus culture production. Significant correlation between studied species callus weight and regeneration capacity was found out. As a result of the study, a new “method of photosynthetic active wheat callus culture production” was developed.

Thus, tetraploid wheat species, and especially the *T. dicoccum*, can be successfully used as sources of drought resistance in interspecific crosses in wheat breeding programs.

The results of present research are widely presented in multi-author monography and some scientific articles. It may be applied in plant physiology, genetics, biotechnology, and plant breeding. The developed approaches may be used for limit stage of production process revealed as well as for screening of wheat selection collection for drought-tolerant forms. These methods provide and sustain morpho-physiological, anatomic, physiological, and biotechnological markers at different stages of ontogenesis in callus cultures in vitro.

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Conflict of interest

The authors declare that they have no conflict of interests.

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