Morphophyziological and Anatomical Characteristics of Leaves in Accessions of Wild Einkorn (*Triticum boeoticum* Boiss.)

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Abstract— *The aim of this study was to assess the degree of variation between 32 accessions of wild einkorn (Triticum boeoticum* Boiss.*) on the basic morphophysiological and anatomical characteristics of the flag and subflag leaves. The experiment was carried out during 2016 – 2017 growing seasons in the randomized block design in four replications and 10 m^2 plot size. Significant differences among the accessions for all studied characters were recorded. The epidermis of the studied 32 accessions was constructed by strongly elongated prosenhyme cells with flexous walls. The stomatas were with oval to elliptic shape, about 1.5 times longer than wide. The most variable character was the total chlorophyll content. Accessions with numbers B6E0416, B6E0413, B6E0398 and B6E0392 had the largest amount of chlorophyll pigments exceeding the average standard almost twice. The water-to-biomass ratio in the flag leaf was the greatest for B6E0378, B6E0389 and B6E0401, while for the subflag leaves B6E0379, B6E0401 and B6E0385 were with the highest amount of water per unit of dry mass. The correlation between intensity of transpiration and the fresh and dry mass of leaves were slightly negative for flag leaf and slightly positive for subflag leaf. The water content of the subflag leaf had a stronger influence on the morphophysiological parameters compared to the water content of the flag leaf. PC-analysis grouped accessions according to similarity on the basis of investigated morphophysiological and physiological characters in two components in the factor plane.*

Keywords—wild einkorn, anatomy of leaf, morphophysiological characters of leaves, correlation, PC-analysis.

I. INTRODUCTION

Drought is known to limit plant productivity in many regions of the world (Chartzoulakis *et al*., 2002). Water deficit is also known to alter a variety of biochemical and physiological processes ranging from photosynthesis to protein synthesis and solute accumulation (Hu & Schmidhalter, 1998). Photosynthesis is the key process of primary metabolism, and its capacity can influence plant performance and productivity (Lawlor & Tezara, 2009; Pinheiro & Chaves, 2011). The extent to which photosynthetic capability is maintained during periods of water stress and the ability of rapid recovery of photosynthesis after rewatering may play an important role in plant adaptation to drought environments. In order to preserve photosynthesis under drought conditions, plants have evolved physiological processes to maintain to some extent tissue turgor and stomatal opening (Chartzoulakis *et al*., 2002). Stomata regulate CO2 diffusion into, and water diffusion out of, plant leaves (Chaves *et al*., 2002). Under water-deficit conditions, plants close stomata to prevent major water loss; this, consequently, reduces photosynthesis via decreased influx of CO2 (Pinheiro & Chaves, 2011). In the long-term response to water deficit, stomatal conductance can be influenced by leaf anatomical traits such as stomatal density and size, which can vary to acclimate to the environment (Xu and Zhou, 2008; Franks & Beerling, 2009; Ouyang *et al*., 2017). Leaf anatomical characteristics are considered the true indicators of stress influence (Aberentthy *et al*., 1998). Number of epidermal cells decreases progressively with the increase in water stress, but number of stomata decreases slightly (McCree & Davis, 1974). Drought resistant wheat genotypes had greater stomatal frequency than susceptible genotypes in drought conditions, and drought susceptible genotypes had higher frequency than drought resistant in irrigated conditions (Nayeem, 1989). Thickness of leaf, cuticle, epidermis, hypodermis, and number of stomata generally increased under water stress while the number of hair and stomatal length decreased (Hameed *et al*., 2002).

Wild wheat species have great potential as a source of genetic traits to improve the drought resistance of wheat cultivars because wild wheat species are highly tolerant to drought stress (Budak *et al.*, 2013). The wild wheat species, *Triticum boeoticum* Boiss., is more tolerant to drought than other wheat relatives, such as *Triticum dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf., *Triticum araraticum* Jakubz. and common wheat cultivars (Sultan *et al.*, 2012; Hui Liu *et al.*, 2015).

There is little information regarding to the variation of morphophysiological and anatomical characteristics leaves of *Triticum boeoticum* Boiss. The importance of the internal exposed surface of the leaves for plant activity is well recognized, especially in certain phenological stages of development of the crop, i.e. the critical period (from 20 days before flowering to10 days after flowering) and the grain filling period. These phases are of great importance for the generation of number of grains and its final weight, respectively. Water, oxygen and carbon dioxide are exchanged through this surface and the rates of most cellular activities depend on this exchange (Filgueira & Golik, 2003).

The aim of this study was to assess the degree of variation between 32 accessions of wild einkorn (*Triticum boeoticum* Boiss.) on the basic morphophysiological and anatomical characteristics of the flag and subflag leaves as indicators of dry resistance.

II. MATERIAL AND METHODS

Field experiment

The study was conducted in the experimental field of IPGR – Sadovo, in the period 2016 - 2017 with 32 accessions from the ex situ collection, belonging to the species *Triticum boeoticum* Boiss. The experiment was carried out in the randomized block design in four replications and 10 m^2 plot size, after the predecessor peas. Normal agronomic and cultural practices were applied to the experiment throughout the growing seasons. Type of bush (at tillering), ligule-presence, auricles -length, leaf-flag attitude (at the beginning of heading), and leaf pubescence were determined according to international descriptor for genera *Triticum* (Anonymous, 1984). In phase of end of heading were made biometric measurements of the following parameters: length and width of flag and subflag leaves. From each accession, 30 leaves were collected for biometrical measurements. Leaf area was calculated by the formula of Kerin *at al.* (1997), Chanda *et al.* (2002) and Berova *et al.* (2004):

$A=k*l*b$, where:

k- coefficient, different for each genera (0.65);

l - length of the leaf along the central vain;

b - maximum leaf width.

Laboratory experiment

Fresh (FW, g) and dry weight (DW, g) of flag and subflag leaves are determined using a precision electronic analytical balance OHAUS AS60-USA. Dry weight of leaves is determined by drying the leaves at 104ºC for 1 hour or until reaching a constant mass in three consecutive measurements (Beadle, 1993).

Water content (WC) in flag and subflag leaves is determined by calculating the water to dry weight ratio-gH2O/gDW.

Intensity of the transpiration (T) in flag and subflag leaves is determined by method of Ivanov *et al.* (1950) with modifications by Georgiev & Valchev (1991).

Chlorophyll content meter (CCM-200, Opti-science, Inc., NH, USA) is used to measure the total chlorophyll count in the leaves.

The microscopic observations of the epidermal cells of the 32 accessions were made with light microscope Olympus CX22LED, with total magnification 400. The following characters of flag leaves are analyzed: length and width of stomata and length and width of epidermal cells.

Statistical analyzes

The mean data from all characters were used to analyze the variance according to Lydansky (1988). LSD test was carried out to explore the significance of differences between mean standard and respective accession in the data set.

Phenotypic correlations were calculated by using of phenotypic variances and covariance. The phenotypic correlations thus calculated were tested for significance (Lydansky, 1988).

PC-analysis was applied to group accessions according to similarity on the basis of morphophysiological and physiological characters in two components in the factor plane.

Statistical analyses were performed using the statistical program SPSS 19.0.

III. RESULTS AND DISCUSSION

Leaf anatomy

For genus *Triticum* is typical the isolateral leaf structure, stomata are situated at both sides of leaf (amphystomatic leaf) and absence of accompanying cells – stomata of anomocyte type. It is considered that the bigger number of accompanying cells is typical for the evolutionary primitive plant families of monocotyledons (Ninova, 1995), so the absence of these cells in genus Triticum is a sign of evolutionary higher stage (Uzundzhalieva *et al.*, 2017).

The epidermis of the studied 32 accessions from *Triticum boeoticum* Boiss. species was constructed by strongly elongated prosenhyme cells with flexous walls (Fig.1). The cell length varied from 444.71 µm for B6E0414 to 1468.14 µm for (B6E0412A). The length of epidermal

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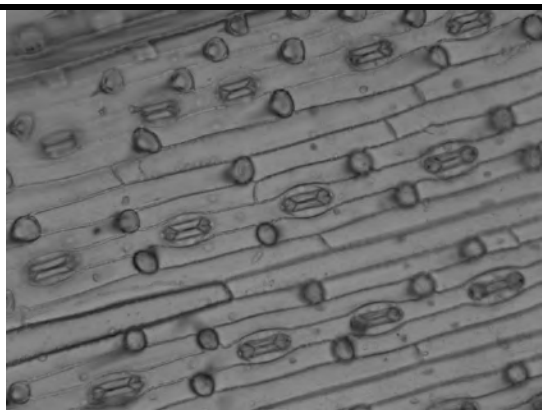
cells in seventeen of accessions was above 1000 µm. The width of the epidermal cells in five of the studied samples had proven differences in compare with the mean standard of the trial. The smallest cells had B6E0414 – 444.71 µm long and 34 µm wide (Table 1). The stomatas were with oval to elliptic shape (Fig.1), about 1.5 times longer than wide. The average length and width of stomata was respectively 381.14 µm and 235 µm. The longest stomata had B6E0397 (449 µm), B6E0410 (466 µm) and B6E0413 (470.14 µm), while the widest stomata had B6E0401 (279.56), B6E0398 (279.57 µm), B6E0380 (280.86 µm), B6E0410 (293.71 µm), B6E0390 (297.71 µm), B6E0400 (347.57 µm). The values of coefficient of variation (CV, %) were above 20% for length of epidermal cells and width of stomata (21.06% and 21.30%, respectively) (Table 1).



Фиг.1 Epidermal cells in *Triticum boeoticum* Boiss.

Table.1: Anatomical characters of flag leaf in 32 accessions of Triticum boeoticum Boiss.

Accessions	Length of epidermal cells, µm	Width of epidermal cells, µm	Length of stomata, µm	Width of stomata, µm
St	1009.57	172.86	381.14	235.00
B6E0378	796.28	180.71	402.29	264.57*
B6E0379	965.00	180.57	335.00*	246.86
B6E0380	717.14	191.43	371.14	280.86***
B6E0381	1068.00	184.43	392.71	234.57
B6E0382	1040.86	183.86	389.14	245.86
B6E0383	909.28	152.57	405.43	215.14
B6E0385	836.43	182.23	417.14	246.29
B6E0386	1278.43	192.29	444.29**	228.43
B6E0387	1163.00	160.29	385.71	242.29
B6E0388	732.00	180.00	359.29	194.14**
B6E0389	1113.57	187.86	439.43**	274.86**
B6E0390	761.28	209.29**	403.29	297.71***
B6E0392	984.00	167.57	386.00	263.71*
B6E0397	1164.57	232.71***	449***	159.43***
B6E0398	755.14	205.57**	346.00	279.57***
B6E0399	1049.00	171.00	347.29	222.57
B6E0400	1120.71	209.00**	446.86**	347.57***
B6E0401	1338.14	180.86	396.43	279.56***
B6E0401A	1190.00	178.71	379.00	261.57*
B6E0402B	862.86	169.29	314.14**	215.86
B6E0405	1078.71	186.29	392.86	224.86
B6E0410	1145.14	186.71	466.00***	293.71***
B6E0412A	1468.14**	185.14	346.71	238.14
B6E0412B	1082.86	151.14	390.86	224.29
B6E0413	1062.86	150.57	470.14***	188.43***
B6E0414	444.71*	34.00***	122.43***	70.00***
B6E0415	836.86	141.57*	318.29**	203.29*
B6E0416	1176.71	150.14	404.00	186.86***
B6E0418B	1289.57	162.57	363.29	183.71***
B6E0420	1002.86	121.43***	381.57	242.71
B6E0421	889.43	164.29	343.71	190.14***
B6E0423	974.43	184.71	387.86	266.29*
LSD0.5	335.04	23.87	39.64	24.33
LSD0.01	442.18	31.50	52.31	32.11
LSD0.001	568.03	40.47	67.20	41.25
CV, %	21.06	19.40	16.25	21.30

*p<0.05, **p<0.01, *** p<0.001

Morphological characters

Accessions from analyzed wild einkorn (*Triticum boeoticum* Boiss.) are characterized with prostrate type of bush (>70º), present of legule and medium size of auricles. The leaves are hairy, which is a typical characteristic for accessions from species *Triticum boeoticum* Boiss. (Empilli *et al.*, 2000). Leaf-flag attitude was dropping (91-135º) in 24 of accessions, while in

B6E0397, B6E0401, B6E0412A and B6E0423 it was semi-upright (15-45º). Accessions with number B6E0379, B6E0390, B6E0392, B6E0416 had horizontal leaf-flag attitude.

1. Flag leaf

1.1. Morphophysiological characters

In Table 2 are presented the results from the biometric analysis of the morphophysiological characters of the flag leaves - length (L, cm) and width (W, cm) of leaves, leaf area (LA, cm^2), fresh weight of flag leaf (FW, g), dray weight of flag leaf (DW, g). Length of the flag leaf varied between 6.83 cm and 15.33 cm. Accessions with numbers B6E0416 and B6E0413 are characterized with proven the longest leaves, respectively 15.33 cm and 15.17 cm, while B6E0380 and B6E0423 were proven the shortest (7.03 cm and 6.83 cm). The average width of the flag leaf was 0.52 cm. In B6E0389 (0.73 cm) and B6E0416 (0.80 cm) are recorded the largest values for this characters against the average experience standard at level of statistical significance $p \leq 0.01$ and $p \leq 0.001$. With the largest leaf area were B6E0416 (8.15 cm^2), B6E0389 (5.66 cm^2) and B6E0413 (5.57 cm^2), while with the smallest B6E0423 (1.50 cm^2) and B6E0380 (1.68 cm^2). The fresh weight of flag leaf of samples B6E0416, B6E0413 and B6E0400 was the largest and exceeds the average standard value of about 38.0%. Accession B6E0423 had the lowest fresh mass value that was below the average standard. In the morphophysiological indicator dry biomass, samples B6E0416, B6E0413 and B6E0400 accumulate the most biomass, the value of which was about 30.0% above the average standard. On the other hand, sample B6E0423 had the lowest biomass accumulation, respectively 0.0149 cm^2 (Table 2).

1.2. Physiological characters

The water-to-biomass ratio shows the water content in the flag leaf. This ratio was the greatest for samples B6E0378 (1.84), B6E0389 (1.78) and B6E0401 (1.73). In accession with number B6E0389, good hydration is combined with a large leaf area, whereas samples B6E0378 and B6E0401 had leaf area below the average standard. The lowest water content had B6E0401A, for which one of the smallest leaf area was also measured (Table 2).

Transpiration refers to evaporation from plant tissue. The process is quite passive, driven by the water vapor difference between the stomatal cavity (or intercellular space) and the surrounding air. When stomata are open, almost all transpiration occurs through the stomata, but plants also transpire through the cuticular layer, which is referred to as cuticular transpiration (Kubota, 2016). The morphological characteristics of the leaves and the plant as a whole, as well as the factors of the environment, influence the intensity of the transpiration (Tzvetkov & Anev, 2017).

The highest intensity of transpiration was reported for B6E0380 (0.540 $mg/cm^2/1$ min), B6E0388 (0.465 $mg/cm^2/1$ min) and B6E0423 (0.440 $mg/cm^2/1$ min), with B6E0380 having leaf area, water content and dry mass of leaf below the value of the average standard. For the remaining accessions B6E0388 and B6E0423, similar values were observed for leaf areas and dray weight of flag leaf, indicating low transpiration efficiency in these accessions. For samples with the highest dry mass of leaf, water content and leaf area values, the intensity of transpiration was about the average standard (Table 2).

The chlorophyll content is an important experimental parameter in the agronomy and in the plant biology research (Lamb *et al.*, 2012). It shows alteration depending on many edaphic and climatic factors such as salt stress, light, water stress, air pollution, fertilizing and also it shows alteration depending on time in vegetation period (Sevik *et al.*, 2012). In our experiment the amount of chlorophyll expressed as a total chlorophyll content index (CCI) ranged from 2.51 to 20.89. One of the reasons for the strong variation in the value of CCI is the difference in time of occurrence of the seed filling phase of the accessions as well as its duration. Accessions with numbers B6E0416, B6E0413, B6E0398 and B6E0392 had the largest amount of chlorophyll pigments exceeding the average standard almost twice. The first two samples are characterized with maximum values of the leaf area, fresh and dry mass of leaves (Table 2).

2. Subflag leaf

2.1. Morphophysiological characters

The length of the subflag leaf ranged between 14.03 cm and 27.47 cm, and samples with numbers B6E0383 (27.47 cm), B6E0399 (27.37 cm), B6E0413 (26.83 cm) and B6E0416 (25.93 cm) exceed significantly the average values of the experiment. They had also the largest leaf area. The smallest leaf area had B6E0423, the difference from the standard was almost three times. The width of the subflag leaf ranged from 0.6 cm to 1.1 cm, with the magnitude of range greater than this of the flag leaf. Accessions B6E0399 and B6E0416 had the widest leaves, and samples with numbers B6E0414 and B6E0415 had the narrowest leaves. With the highest fresh mass of the subflag leaves were B6E0399, B6E0386 and B6E0383, their average values being higher than the average standard by 35.0%. B6E0399 and B6E0383 were also indicative of the previous characters. The lowest fresh mass of subflag leaf is reported for B6E0401A. The largest dry mass had B6E0399, B6E0392 and B6E0416. Their values exceed the average standard by more than 30.0%. The lowest dry mass had B6E0401A. It was the only one of all accessions with an average dry mass below 0.05 g (Table 3).

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Table.2: Morphophysiological characters flag leaf in the end of the heading phase and total leaf chlorophyll content index (CCI)

Accessions	FW, g	DW, g	WC, g H_20/g DW	L, cm	W, cm	LA, cm^2	T mg/cm^2/1 min	Total CCI
St	**0.0777**	**0.0322**	**1.42**	**10.35**	**0.52**	**3.67**	**0.306**	**8.28**
B6E0378	0.0580	0.0174	1.84	7.83	0.33*	2.26	0.300	6.54
B6E0379	0.0732	0.0280	1.68	9.56	0.53	3.33	0.330	11.81
B6E0380	0.0497	0.0212	1.37	7.03*	0.37	1.68	0.540*	13.07
B6E0381	0.0771	0.0322	1.48	8.4	0.53	2.94	0.403	8.77
B6E0382	0.0746	0.0341	1.27	10.3	0.53	3.63	0.233	7.70
B6E0383	0.1152	0.0456	1.52	13.1	0.6	4.99	0.289	6.69
B6E0385	0.0797	0.0300	1.65	9.83	0.53	3.47	0.335	12.88
B6E0386	0.1046	0.0432	1.39	11.67	0.63	5.27	0.294	6.89
B6E0387	0.0781	0.0307	1.52	10.63	0.63	4.69	0.295	8.80
B6E0388	0.0645	0.0254	1.59	8.26	0.43	2.32	0.465	3.57
B6E0389	0.1014	0.0362	1.78	11.5	0.73**	5.66*	0.276	3.31
B6E0390	0.101	0.0387	1.66	11.33	0.53	4.02	0.368	5.84
B6E0392	0.093	0.0366	1.53	10.2	0.5	3.32	0.388	16.33*
B6E0397	0.056	0.0242	1.32	8.93	0.47	2.78	0.372	11.90
B6E0398	0.067	0.0268	1.50	9.29	0.5	3.02	0.299	18.33*
B6E0399	0.099	0.0418	1.38	11.33	0.67	5.31	0.376	12.79
B6E0400	0.1209	0.0480	1.52	12.93	0.53	4.47	0.333	3.63
B6E0401	0.0797	0.0293	1.73	10.4	0.53	3.63	0.395	2.93
B6E0401A	0.0409	0.0208	1.00	8.03	0.33*	1.76	0.285	2.51
B6E0402B	0.0566	0.0234	1.40	8.83	0.3**	1.74	0.307	6.13
B6E0405	0.0779	0.0332	1.35	9.53	0.47	2.91	0.129	3.43
B6E0410	0.0809	0.0361	1.23	10.5	0.53	3.68	0.106	3.39
B6E0412A	0.0732	0.0308	1.36	10	0.53	3.53	0.275	3.52
B6E0412B	0.0701	0.0296	1.35	11.67	0.53	4.08	0.181	4.07
B6E0413	0.1212	0.0537*	1.26	15.17***	0.57	5.57	0.355	15.14
B6E0414	0.0437	0.0191	1.29	9.86	0.43	2.77	0.135	14.46
B6E0415	0.0631	0.0311	1.02	11.38	0.47	3.71	0.215	7.27
B6E0416	0.1256	0.0578*	1.16	15.33***	0.8***	8.15***	0.271	20.89**
B6E0418B	0.0755	0.0329	1.31	10.97	0.57	4.26	0.273	4.71
B6E0420	0.0616	0.0252	1.43	10.03	0.43	2.94	0.359	2.51
B6E0421	0.0730	0.0309	1.36	10.7	0.57	3.94	0.179	6.86
B6E0423	0.0310	0.0149	1.15	6.83*	0.33*	1.50	0.440	11.24
LSD0.5	0.050	0.019	0.579	2.76	0.16	1.98	0.226	7.915
LSD0.01	0.067	0.025	0.771	3.66	0.21	2.63	0.301	10.446
LSD0.001	0.086	0.032	0.998	4.72	0.27	3.39	0.389	13.419
CV, %	30.23	30.66	14.12	18.82	21.62	37.61	31.28	59.53

*$p<0.05$, **$p<0.01$, *** $p<0.001$

Length of leaf (L, cm), and width of leaf (W, cm), leaf area (LA, cm^2), fresh weight of leaf (FW, g), dray weight of leaf (DW, g), water content (WC, g H_20/g DW), transpiration (T, mg/cm^2/1 min), total leaf chlorophyll content index (CCI), coefficient of variation (CV, %)

2.2. Physiological characters

Accessions with numbers B6E0379, B6E0401 and B6E0385 were with the highest amount of water per unit of dry mass. B6E0423 had the smallest water content. With the lowest water content per unit of dry mass was B6E0423, the difference with the leading accessions was about 1 g. For this sample, the lowest values for leaf width and leaf area were also reported. The highest intensity of transpiration was found in samples B6E0381, B6E0388 and B6E0398 and respectively the lowest intensity in accession B6E0401A. In B6E0381 and B6E0398, strong transpiration was combined with relatively high values of fresh and dry mass. Compared to them, in sample with number B6E0388, transpiration was ineffective. In B6E0401A there was, also an ineffective transpiration (Table 3).

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Table. 3: Morphophysiological characters of subflag leaf in the end of the heading phase

Accessions	FW, g	DW, g	WC, g H_20/g DW	L, cm	W, cm	LA, cm	T, mg/cm^2/1 min
St	0.227	0.083	1.728	20.23	0.84	11.37	0.231
B6E0378	0.2365	0.0938	1.41	19.73	0.83	11.88	0.195
B6E0379	0.2478	0.0808	2.52*	19.57	0.97	12.4	0.209
B6E0380	0.1968	0.0677	1.90	15.67	0.73	7.72	0.275
B6E0381	0.2468	0.0804	2.07	18.33	0.73	8.93	0.332
B6E0382	0.2537	0.0899	1.76	23.77	0.8	12.36	0.259
B6E0383	0.3096	0.1095	1.81	27.47**	0.9	16.07*	0.285
B6E0385	0.2745	0.0862	2.14	20.77	0.97	13.08	0.238
B6E0386	0.3209	0.1112	1.88	22.73	0.97	14.37	0.316
B6E0387	0.2599	0.0899	1.87	21.8	0.97	13.76	0.257
B6E0388	0.1891	0.0655	1.93	15.9	0.83	8.79	0.329
B6E0389	0.2489	0.0888	1.78	20.9	1.03**	13.96	0.217
B6E0390	0.2401	0.0791	2.07	19.77	0.97	12.49	0.222
B6E0392	0.3397	0.1251	1.72	23.33	1*	15.17	0.254
B6E0397	0.2021	0.0686	1.94	19.33	0.97	12.2	0.260
B6E0398	0.2069	0.0730	1.93	14.72*	0.8	7.85	0.317
B6E0399	0.4006*	0.1482**	1.72	27.37**	1.1***	19.48***	0.175
B6E0400	0.3017	0.1049	1.84	23.63	0.83	13.18	0.285
B6E0401	0.2183	0.0674	2.21	19.23	0.87	11.1	0.253
B6E0401A	0.1061	0.0443	1.36	16.23	0.6***	6.33*	0.082*
B6E0402B	0.1315	0.0506	1.57	14.03*	0.67*	6.11*	0.171
B6E0405	0.2924	0.1080	1.78	21.2	0.87	12.39	0.179
B6E0410	0.1973	0.0832	1.50	19.5	0.77	10.02	0.166
B6E0412A	0.2502	0.0935	1.71	21.53	0.93	13.39	0.217
B6E0412B	0.1775	0.0720	1.47	22.37	0.63**	9.13	0.156
B6E0413	0.2508	0.1091	1.23	26.83**	0.8	13.98	0.235
B6E0414	0.1466	0.0573	1.55	19.7	0.63**	8.14	0.207
B6E0415	0.1192	0.0533	1.22	18.67	0.63**	7.71	0.116
B6E0416	0.2780	0.1134	1.48	25.93*	1.07**	18.03**	0.202
B6E0418B	0.1668	0.0675	1.48	17.37	0.97	10.93	0.166
B6E0420	0.1472	0.0540	1.72	17.5	0.7*	7.96	0.264
B6E0421	0.1472	0.0540	1.72	18.33	0.83	9.92	0.246
B6E0423	0.1222	0.0610	1.11	14.10*	0.53***	4.99**	0.299
LSD0.5	0.130	0.046	0.758	4.86	0.14	4.01	0.134
LSD0.01	0.173	0.061	1.009	6.45	0.19	5.33	0.178
LSD0.001	0.224	0.079	1.307	8.31	0.24	6.87	0.231
CV, %	35.07	33.05	18.57	21.75	20.72	34.31	29.50

*p<0.05, **p<0.01, *** p<0.001

Length of leaf (L, cm), and width of leaf (W, cm), leaf area (LA, cm^2), fresh weight of leaf (FW, g), dray weight of leaf (DW, g), water content (WC, g H_20/g DW), transpiration (T, mg/cm^2/1 min), coefficient of variation (CV, %)

Correlation between investigated characters

Correlations between the morphophysiological and physiological characters reported for the flag and the subflag leaves were with moderate and strong positive values with a proof of up to 1% (table 4 and table 5).

There were some differences in the calculated correlation between the morphophysiological and physiological indicators of both types of leaves. The relationship between intensity of transpiration with the fresh and dry mass of leaves, were slightly negative for flag leaf and slightly positive for subflag leaf, respectively. The water content of the subflag leaf had a stronger influence on the morphophysiological parameters compared to the water content of the flag leaf, with significant at $p \leq 0.05$. For both types of leaves, the CCI value affected positively on the most of the characters, with stronger impact on the flag leaf (table 4 and table 5).

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Table. 4: Correlation between morphophysiological and physiological characters of flag leaf

Characters	FW	DW	WC	L	W	LA	T	CCI
FW	1.000	0.971**	0.171	0.886**	0.654**	0.862**	-0.083	0.477**
DW		1.000	-0.061	0.915**	0.670**	0.889**	-0.164	0.501**
WC			1.000	-0.081	0.036	-0.040	0.353**	-0.059
L				1.000	0.583**	0.861**	-0.261	0.408*
W					1.000	0.894**	-0.262	0.423**
LA						1.000	-0.268	0.538**
T							1.000	0.068
CCI								1.000

*p<0.05, **p<0.01, *** p<0.001

FW - fresh weight of leaf, DW - dray weight of leaf, WC - water content in the leaf, L- length of leaf, W - width of leaf, LA - leaf area, T - Intensity of the transpiration in the leaf, CCI - Chlorophyll content index.

Table. 5: Correlation between morphophysiological and physiological characters of subflag leaf

Characters	FW	DW	WC	L	W	LA	T	CCI
FW	1.000	0.948**	0.407*	0.774**	0.758**	0.886**	0.271	0.308
DW		1.000	0.119	0.835**	0.699**	0.896**	0.123	0.330*
WC			1.000	0.032	0.418*	0.231	0.481**	0.036
L				1.000	0.544**	0.872**	-0.031	0.193
W					1.000	0.876**	0.088	0.315
LA						1.000	0.025	0.303
T							1.000	0.125
CCI								1.000

*p<0.05, **p<0.01, *** p<0.001

FW - fresh weight of leaf, DW - dray weight of leaf, WC - water content in the leaf, L- length of leaf, W - width of leaf, LA - leaf area, T - Intensity of the transpiration in the leaf, CCI - Chlorophyll content index.

Principal component analysis (PC-analysis)

PC-analysis was applied to group accessions according to similarity on the basis of investigated morphophysiological and physiological characters in two components in the factor plane. The values of the two components to each of the study parameters for flag and subflag leaves were calculated empirically (Table 6). The analysis shows that the first component explains 63.16 % of the total variation in the trial with flag leaves and 62.16% of the total variation in the experiment with subflag leaves, the second - 20.57 % and 21.11%, respectively for the experiments with flag and subflag leaves. Two factors explain total 83.73 % of the variation in the experience with flag leaves and 83.27% in the experience with subflag leaves. First factor had an important role to justify alteration of FW, DW, L, W and LA, while second factor was in positive correlation with WC and T (Table 6).

Distribution of evaluated accessions in the coordinate system of PC1 and PC2, presents the grouping of accessions according to similarity of traits: FW, DW, L, W, LA, WC and T both for experiment with flag leaf (in left) and experiment with subflag leaf (in right) (Fig. 2). The accessions grouped in the upper left quadrants had positive values for PC1 and negative values for PC2 (high FW, DW, L, W, LA and low WC and T). The samples classified in the upper right quadrants had positives values for both factors (PC1 and PC2). Accessions in the below left quadrants had respectively negative values for both factors. The samples in the below right quadrants are characterized with negative values for PC1 and positive values for PC2. Some of the accessions are separated as "detached" from other. For the both experiments these accessions were B6E401A, B6E415 and B6E0388. B6E401A and B6E415 had low values of all characters included in the factor analyses. B6E0388 is characterized with high value of T and moderate value of WC in the both types of analyzed leaves. B6E0416 is characterized with the highest values of L, W, LA, FW, DW as well as with low values of WC and T of the flag leaf, while B6E399 for the subflag leaf.

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Table. 6: Factor analysis of traits using principal components analysis in the trials with flag and subflag leaves in 32 genotypes from Triticum boeoticum Boiss.

Characters	Rotated Component Matrix in the trial with flag leaf		Rotated Component Matrix in the trial with subflag leaf	
	Components		Components	
	1	2	1	2
FW - fresh weight of leaf,	0,95	0,17	0,92	0,30
DW - dray weight of leaf	0,95	-0,06	0,95	0,05
WC - water content in the leaf	0,01	0,84	0,15	0,87
L- length of leaf	0,92	-0,23	0,91	-0,16
W - width of leaf	0,89	0,00	0,79	0,35
LA - leaf area	0,96	-0,11	0,98	0,08
T - Intensity of the transpiration in the leaf	-0,08	0,81	0,01	0,80
Eigen values	4,42	1,44	4,35	1,48
Proportional variance, %	63,16	20,57	62,16	21,11
Cumulative variance, %	63,16	83,73	62,16	83,27

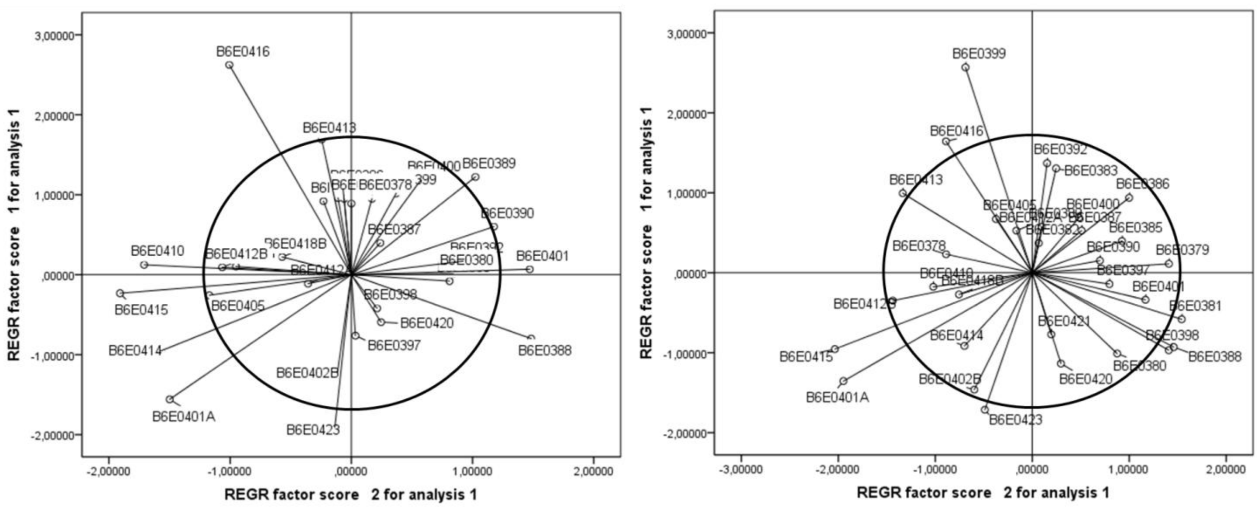

Fig.2: Distribution of evaluated accessions within the factor plane according to similarity of traits: FW - fresh weight of leaf, DW - dray weight of leaf, WC - water content in the leaf, L- length of leaf, W - width of leaf, LA - leaf area, T - Intensity of the transpiration in the leaf, both for experiment with flag leaf (in left) and experiment with subflag leaf (in right)

IV. CONCLUSION

Analysis of variance showed highly significant differences among the accessions for all anatomical, morphological and physiological characters included in the study for the flag and subflag leaves. The most variable character was the total chlorophyll content. B6E0416 and B6E0413 are characterized with the largest leaf area, fresh and dry mass of the flag leaves and high total chlorophyll content. Low transpiration efficiency of flag leaf was detected for B6E0380, B6E0388 and B6E0423. An ineffective transpiration of subflag leaf had number B6E0388 and B6E0401A. The correlation between intensity of transpiration and the fresh and dry mass of leaves were slightly negative for flag leaf and slightly positive for subflag leaf. The water content of the subflag leaf had a stronger influence on the morphophysiological parameters compared to the water content of the flag leaf. The total chlorophyll content in the leaves expressed through CCI value affected positively on the most of the morphophysiological and physiological characters, with stronger impact on the flag leaf. PC-analysis grouped accessions according to similarity on the basis of investigated morphophysiological and physiological characters in two components in the factor plane. First factor had an important role to justify alteration of fresh weight of leaf, dray weight of leaf, length of leaf, width of leaf, and leaf area, while second factor was in positive correlation with water content in the leaf and intensity of the transpiration.

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