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Tolerance of the photosynthetic apparatus in recombinant lines of wheat adapting to water stress of varying intensity

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Abstract

The stress tolerance index (STI) of leaf photosynthetic parameters was analysed in recombinant introgression lines of spring wheat (*Triticum aestivum* L.) grown under water stress of varying intensity in the simulated conditions of soil and soil-atmospheric drought. STI for the chlorophyll content was >1 regardless of experimental conditions. Carotenoids content increased only when soil drought occurred. Maximum quantum yield of PSII photochemistry was the most stable and stress-resistant parameter. Minimal fluorescence yield of the dark-adapted state was only sensitive to soil-atmospheric drought. Nonphotochemical quenching decreased under water stress, while parameters of the fast light curve based on chlorophyll fluorescence increased proportionally to the level of the stress load. We believe that these parameters are the most sensitive to the changes in the water supply of wheat plants, and are convenient for the rapid and noninvasive assessment of the wheat photosynthetic apparatus state under drought conditions.

Additional key words: antioxidant enzymes; carotenoids; chlorophyll content; chlorophyll fluorescence; drought; gas exchange.

Introduction

Photosynthesis is a sophisticated multistage process involving the synthesis of complex carbohydrates from CO_2. During the photo-phase of this process, water molecules donate the electrons necessary to transform $NADP^+$ into NADPH. For this reason, photosynthesis is extremely sensitive to water stress caused not only by a low soil water content, but also by the water vapour deficiency in the air. The photosynthetic response of a plant to water stress is complex and depends on the intensity and duration of the stress as well as on the water stress combined with other stress factors (Chaves *et al.* 2009, Ashraf and Harris 2013). The response of the photosynthetic apparatus (PA) to drought was extensively studied and different plants were considered in this respect (Ashraf and Harris 2013, Cicevan *et al.* 2016, Mathobo *et al*. 2017), including strategic agricultural crops such as wheat. For the latter, gas exchange and chlorophyll (Chl) fluorescence were shown to depend not only on environmental conditions, but also, essentially, on the genotype (variety) (Liu *et al*. 2006, Zivcak *et al*. 2008, 2013; Keyvan 2010, Lonbani and Arzani 2011, Wang *et al*. 2016, Li *et al*. 2017). The shoot biomass and leaf photosynthetic parameters were measured in recombinant introgression lines (RILs) Chinese Spring/Synthetic 6x (CS/Syn), grown under optimal conditions of water supply and water stress of varying intensity to map quantitative trait loci (QTL) related to the functioning of PA in the genome of common wheat (Osipova *et al*. 2016, Permyakova *et al*. 2017). However, the comparative analysis of the photosynthetic parameters tolerance, observed during the period of adaptation of wheat to water

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Abbreviations: BM − shoot biomass; Chl – chlorophyll; Car – carotenoids; DM – dry mass; E – transpiration rate; ETR_{160} – electron transport rate at 160 μmol(photon) m^{-2} s^{-1}; ETR_{max} – maximum electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_0' – minimal fluorescence yield of the light-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_t – stationary Chl fluorescence; F_V/F_m – maximum quantum yield of PSII photochemistry; F_V/F_0 – the contribution of the light reactions to primary photochemistry; g_s – stomatal conductance; lk – intensity of illumination, expressing the beginning of PAR saturation; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; PA – photosynthetic apparatus; q_N – nonphotochemical quenching coefficient; RILs – recombinant introgression lines; R_{fd} – vitality index; STI – stress tolerance index; SD – soil drought; SAD – soil-atmospheric drought; SOD – superoxide dismutase; APX – ascorbate peroxidase; DHAR – dehydroascorbate reductase; GR – glutathione reductase; TChl – total chlorophyll; WUE – water-use efficiency (= P_N/E); Φ_{PSII} – effective quantum yield of PSII photochemistry.
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stress of different intensity, remained untouched in the published studies just cited.

A convenient noninvasive method for assessing the stability of PA including drought is based on measuring the fluorescence of Chl. The applicability of various parameters, obtained with the help of this method (taking into account their physiological values), for rapid screening of the resistance of plants to unfavourable environmental conditions, is actively discussed in the literature (Lichtenthaler *et al*. 2005, Lazár 2015, Goltsev *et al.* 2016, Kalaji *et al*. 2017). The dissipation of excess light energy can occur in various ways. As a rule, the nonphotochemical quenching of fluorescence increases under stress, but Zivcak *et al*. (2013) showed that for winter wheat, the cyclic path of electrons around PSII and PSI is the main way how to dissipate excess energy during drought. We assumed that the fast light curve based on Chl fluorescence indicators can be the most sensitive to water stress in wheat and can serve as a convenient tool for screening wheat genotypes for drought resistance in various breeding programs. The aim of this work was a large-scale verification of this hypothesis under conditions of water stress with different intensity using a set of wheat RILs CS/Syn, which allowed analysis of the variability in responses to water stress.

Materials and methods

Plant materials and experimental conditions: In our work, we used the set of genome D RILs created by means of crossing the Chinese Spring wheat and the synthetic hexaploid Synthetic 6x (Pestsova *et al.* 2006). Each line carried a certain area of introgression from the wild grass of *Aegilops tauschii* Coss on the genetic background of the selection wheat, Chinese Spring. Thus, RILs allowed studying the variability of photosynthetic parameters in genetically closely related genotypes. In order to reveal the dependence of photosynthetic parameters on the level of stress load, two growth regimes were chosen, which we took as independent experiments. During the first independent experiment, 71 lines were grown in the greenhouse of Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch of the Russian Academy of Sciences (SIPPB SB RAS) under a 16-h photoperiod under conditions of natural daylight with a 2-h illumination in the morning and in the evening at around 600 μmol(photon) m^{-2} s^{-1}. Temperature fluctuations were 21/15°C, and the ones of relative air humidity were about 20/30% during day/night. During the second independent experiment, 79 lines were grown under controlled conditions in a climatic chamber *CLF Plant Master* (*CLF Plant Climatic GMBH*, Wertingen, Germany), and a 16-h photoperiod was maintained at 600 μmol(photon) m^{-2} s^{-1}. Temperature fluctuations were 23/16°C during day/night and the relative air humidity was 60%. In each experiment, the plants were grown in a 1:1:1 of humus:sand:peat mixture and two variants of water supply were used – an optimal one and one of water stress. In one pot, the plants were kept well-watered, while in the other pot, water was withheld from the three-leaf stages until the soil moisture content had fallen to 30% of saturation. The moisture content of the soil for the well-watered control plants was maintained at 60% saturation. Soil moisture status was monitored three times per week by weighing the pots, and adjusted when necessary. The conditions of the first independent experiment were distinguished by a low air humidity, and a water stress variant in this case was indicated as soil-atmospheric drought (SAD), while in the second experiment it was identified as soil drought (SD). Twenty-four hours after the Chl fluorescence was measured, flag leaf material was frozen with liquid nitrogen and was kept at −70°C until the time of the analysis of Chl and carotenoids (Car) contents.

Chl and Car contents: A 50-mg aliquot of frozen flag leaf tissue was homogenized in 3 mL of 80% acetone with 10 mg of $CaCO_3$. The homogenate was made up to 10 mL with 80% acetone, then centrifuged at 2,000 × *g* for 10 min. A 3-mL aliquot of the supernatant was used to measure absorbance at 440.5, 648, and 664 nm using a *Hitachi U-1100* spectrophotometer (*Hitachi Ltd*., Tokyo, Japan). The contents of Chl *a*, Chl *b*, Chl (*a*+*b*), and Car were calculated according to the extinction coefficients given by Wettstein *(*1957). Pigment contents were expressed as pigment contents per gram dry mass of leaves [mg g^{-1}(DM)].

Enzyme activities: Under conditions of SD, activities of a few enzymes of the water-water cycle were measured, namely: superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11), dehydroascorbate reductase (DHAR, EC 1.8.5.1), and glutathione reductase (GR, EC 1.6.4.2). Enzyme extracts were prepared as described by Osipova *et al.* (2016). Peak activities of DHAR, APX, and GR were determined in batches of three extracts, which were introduced into a flat-bottomed *UV-Star* microplate (*Greiner Bio-One GmbH*, Frickenhausen, Germany); reading were taken using an *Infinite M200 PRO* microplate reader (*Tecan Group Ltd*., Männedorf, Switzerland). Reaction mixtures (200 μL) contained 10 μL of the enzyme extract in all experiments. DHAR was assayed in 50 mM potassium phosphate buffer, pH 7.0, 0.2 mM dehydroascorbate (*Sigma-Aldrich*, USA), and 2.5 mM reduced glutathione (*Reanal Private Ltd*., Budapest, Hungary), and its activity was followed by an increase in A_{265} for 1 min (extinction coefficient, 14 mM^{-1} cm^{-1}) (Baier *et al.* 2000). GR activity was determined by following the oxidation of NADPH at 340 nm for 1 min (extinction coefficient of 6.2 mM^{-1} cm^{-1}) in 50 mM potassium phosphate buffer, pH 7.8, containing 0.10 mM NADPH (*Sigma Aldrich*, USA), and 1 mM of oxidized glutathione (*Reanal Private Ltd*., Budapest, Hungary) (de Lamotte 2000). APX activity was determined by following the decrease in A_{290} of an assay mixture containing 50 mM potassium phosphate buffer, pH 7.0, 0.5 mM ascorbic acid (*Sigma Aldrich*, USA) and 0.1 mM H_2O_2 (extinction coefficient, 2.8 mM^{-1} cm^{-1}) (Nakano and Asada 1981). Enzymatic activity was expressed as micromoles of substrate per milligram of protein per min at 25°C. Total SOD activity was measured spectrophotometrically

using an *Infinite M200 PRO* microplate reader and flat-bottomed *Citotest* microplate (www.citotest.com) based on the inhibition of nitroblue tetrazolium (NBT) reduction, following Giannopolitis and Ries (1977). Each 200-μL reaction contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine (*Reanal Private Ltd*., Budapest, Hungary), 2 μM riboflavin (*Reanal Private Ltd*., Budapest, Hungary), 63 μM NBT (*Sigma Aldrich*, USA), 0.1 μM EDTA, and 10 μL of the extract. A unit of SOD (U) was defined as the quantity required to inhibit the reduction of NBT by 50%. Specific enzymatic activity was calculated as U mg^{-1} (protein). Protein content was determined according to Bradford, using BSA (*Sigma Aldrich*, USA) as a standard.

Gas exchange and Chl fluorescence: In each line, the parameters of gas exchange and Chl fluorescence were measured in the middle part of the fully-developed flag leaves at the stage of stalking. Since different lines reached the desired stage non-simultaneously, all measurements were taken within two weeks. Stomatal conductance (g_s), transpiration (E), and net photosynthetic rate (P_N) were measured using a portable gas-exchange system (*LCi Photosynthesis System*, *ADC BioScientific Ltd*., Hoddesdon, England). Water-use efficiency (WUE) was calculated as P_N/E. The measurements of the Chl fluorescence of leaves were carried out using a portable impulse fluorometer *PAM-2500* (*Walz,* Effelrich, Germany). In order to register the minimal fluorescence yield of the dark-adapted state (F_0), we darkened the leaves for 30 min and then illuminated them with modulated measuring light of low frequency (5 Hz) and low intensity (630 nm). The intensity of the Chl fluorescence under conditions of closed reactive centers (F_m) was measured after the exposure of a light impulse of high intensity [25,000 μmol(photon) m^{-2} s^{-1}, 630 nm]. In addition, we calculated the relative rate of electron transport (ETR) provided by PSII, and the effective photochemical quantum yield of PSII (Φ_{PSII}) in the light-adapted state. In order to do this, we carried out the registration of changes in the Chl fluorescence indices observed under the influence of actinic red light exposure 160 μmol(photon) m^{-2} s^{-1}, maintaining photosynthesis. Parameters lk and ETR_{max} were calculated from the Chl fluorescence light curve [PAR range from 0 to 2,000 μmol(photon) m^{-2} s^{-1}]. Measured and calculated Chl fluorescence parameters used in our work are presented in the text table bellow.

Stress tolerance index (STI) as defined by Fernandez (1993) was calculated for all the parameters according to the formula $STI = T_d \times T_c/T^2_c$, where T_d and T_c are values of the attributes under drought conditions and in the control for each genotype, and T_c is the average value of the attribute under controlled conditions for all the genotypes.

Statistical analyses: The main shoot biomass was represented as the average of 8–10 plants per line. In our study, one plant was taken for biological replication. The g_s, E, and P_N were averages of eight biological replicates. Chl fluorescence was measured on the flag leaves of four plants per line. Pigment contents were averages of three biological replicates, each consisting of three analytical replicates. The enzymes activity was measured in three biological replicates, each being run in triplicate. *SigmaPlot v.12.0* (*Systat Software, Inc*., San Jose California, USA, www.sigmaplot.com) was used to calculate means, standard deviations and *Spearman* correlations as well as to perform *t*-tests.

Results

The response of the PA of wheat RILs under conditions of adaptation to SD and SAD: As can be seen from the spider plot (average values of the attributes in percentage under water stress) photosynthesis was limited, mainly by stomatal effects, regardless of the experimental conditions (Fig.1*A,B*; Tables 1S, 2S – *supplements*). Average values of g_s, E, and P_N decreased virtually to the same extent both under SD and SAD conditions compared with the control. Biomass, on the average, was better preserved when the plants were grown in the climatic chamber at an air humidity of 60%. The Chl content approximately equally increased compared with the control under SD and SAD conditions. The plants response to various growing conditions was different in average values of the Car content. Thus, under SAD conditions, the Car content slightly decreased, while under SD conditions, on the contrary, it increased compared with the control, and for this reason, TChl/Car ratio was significantly higher under severe drought. Among parameters of the Chl fluorescence, F_v/F_m, F_m/F_0, and F_v/F_0 were the most stable ones. Under

F_0	Minimum Chl fluorescence from a dark-adapted leaf (PSII centers open)
F_m	Maximum Chl fluorescence from a dark-adapted leaf (PSII centers closed)
$F_v = F_m - F_0$	Maximum variable Chl fluorescence from a dark-adapted leaf
F_t	Stationary Chl fluorescence
$R_{fd} = [F_m - F_t]/F_t$	Vitality index (Lichtenthaler *et al*. 2005)
$F_v/F_m = [F_m - F_0]/F_m$	Maximum quantum yield of PSII photochemistry
F_v/F_0	The contribution of the light reactions to primary photochemistry
$\Phi_{PSII} = F_m' - F_t/F_m'$	Effective photochemical quantum yield of PSII
$NPQ = [F_m/F_m'] - 1$	Nonphotochemical quenching of chlorophyll fluorescence
$q_N = 1 - [F_m' - F_0']/[F_m - F_0]$	Coefficient of nonphotochemical fluorescence quenching
ETR_{160}	Electron transport rate at 160 μmol(photon) m^{-2} s^{-1}
ETR_{max}	Maximum electron transport rate, μmol(electron) m^{-2} s^{-1}
lk	Intensity of illumination, expressing the beginning of PAR

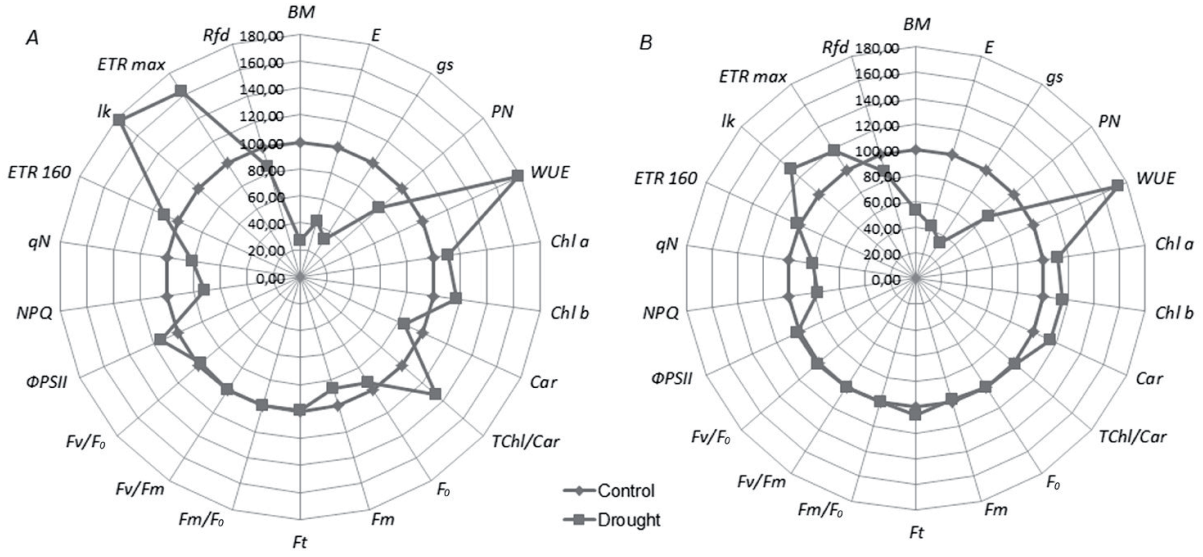

Fig 1. Spider plot shows the relative standard deviation (in %) of the average values of the studied parameters in CS/Syn RILs, which are observed under conditions of soil-atmospheric (*A*, *n* = 71) and soil (*B*, *n* = 79) drought, from the control values of the parameters under optimal watering conditions (control – 100%). BM − shoot biomass; Chl – chlorophyll; Car – carotenoids; ETR_{160} – electron transport rate at 160 μmol(photon) m^{-2} s^{-1}; ETR_{max} – maximum electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_t – stationary Chl fluorescence; F_v/F_m – maximum quantum yield of PSII photochemistry; F_v/F_0 – the contribution of the light reactions to primary photochemistry; g_s – stomatal conductance; lk – intensity of illumination, expressing the beginning of PAR saturation; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; q_N – nonphotochemical quenching coefficient; Rfd – vitality index; TChl – total chlorophyll; WUE – water-use efficiency (= P_N/E); Φ_{PSII} – effective quantum yield of PSII photochemistry.

SAD conditions, F_m decreased significantly when Φ_{PSII} increased, while under SD conditions, these parameters remained unchanged. The parameters of the fast light curve based on Chl fluorescence – lk and ETR_{max} – increased 1.7 times on average under SAD, and 1.2 times under SD. The average values of the parameters, characterizing non-photochemical fluorescence quenching (NPQ and q_N), decreased under stress regardless of the experiment and approximately to the same extent (Fig. 1*A,B*; Tables 1S, 2S).

For a quantitative comparison of PA response to soil and soil-atmospheric water stress, we carried out the analysis of differences between average values of the STI defined by Fernandez (1993). The index was calculated for all the studied attributes in the two independent experiments (Table 1; Tables 3S, 4S - *supplements*). The wide range of changes in STI observed virtually in all the attributes is indicative of the fact that different lines responded differently to the stress conditions of different intensity. STI for the parameters of gas exchange (*E*, g_s, P_N, WUE) of the chlorophyll fluorescence rapid light curve (lk and ETR_{max}) and of NPQ was highly variable in two independent experiments. Additionally, under SAD, STI was significantly different for F_0, F_m, and F_t. Biomass and Car were much more tolerant to SD conditions. Under SAD, the average STI values for F_0, Φ_{PSII}, ETR_{160}, ETR_{max}, and lk were significantly higher. The average STI values for F_v/F_m, F_v/F_0, F_m/F_0, R_{fd}, as well as for NPQ and q_N, remained the same under different water stress intensities.

Under the conditions of soil drought, we identified five lines – 1D 4, 1D 6, 2D 16, 7D 3, and 7D 1b as the most resistant. These lines were characterized by a relatively high stability of the shoot mass (average STI = 0.85) and of the content of photosynthetic pigments (average STI = 1.3–1.5). The average STI values for lk and ETR_{max} in these lines varied around 1. Under more severe conditions of SAD, the line 1D 6 (Tables 3S, 4S) was the most resistant in terms of shoot mass from these five lines.

The interrelation between average STI values for the attributes: Under SAD conditions, STI for the biomass correlated positively with the STI for WUE, ETR_{160}, and lk, and negatively with STI for *E*, g_s, F_0, F_m, F_t, and Φ_{PSII} (Table 2). STI for P_N and WUE positively correlated with STI for lk. The negative correlation of STI for pigments content with STI for F_0, F_m, and F_t could be observed. STI for TChl/Car positively correlated with the STI for F_0. Under SAD, STI for Φ_{PSII} correlated positively with STI for F_0, F_m, F_t, F_v/F_m, F_v/F_0, ETR_{160}, ETR_{max}, and lk, and negatively with STI for NPQ and q_N. STI for NPQ and q_N correlated positively with STI for *E* and g_s, and negatively with STI for F_0, F_m, F_t, and F_v/F_m. Under the same conditions, STI for R_{fd} positively correlated with STI for *E* and g_s, as well as with STI for NPQ and q_N. Finally, STI for R_{fd} correlated negatively with STI for biomass, WUE, ETR_{160}, lk, and ETR_{max}.

Quite a different nature of correlating relationships

Table 1. Average values and the range of variation in the stress tolerance index (STI) for shoot biomass, photosynthetic pigment contents, gas-exchange and Chl fluorescence parameters. BM − shoot biomass; Chl – chlorophyll; Car – carotenoids; ETR_{160} – electron transport rate at 160 μmol(photon) m^{-2} s^{-1}; ETR_{max} – maximum electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_t – stationary Chl fluorescence; F_V/F_m – maximum quantum yield of PSII photochemistry; F_V/F_0 – the contribution of the light reactions to primary photochemistry; g_s – stomatal conductance; lk – intensity of illumination, expressing the beginning of PAR saturation; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; q_N – nonphotochemical quenching coefficient; R_{fd} – vitality index; TChl – total chlorophyll; WUE – water-use efficiency (= P_N/E); Φ_{PSII} – effective quantum yield of PSII photochemistry. ** − significant at $p<0.01$, *** − at $p<0.001$, **** − at $p<0.0001$. Comparisons were made for STI values under different growing conditions. V − the coefficient of variation.

Traits	Greenhouse SAD, *n* = 71			Climatic chamber *CLF PlantMaster* SD, *n* = 79		
	Mean ± SE	Min–Max	V, %	Mean ± SE	Min – Max	V, %
BM	0.3 ± 0.1	0.1 – 0.4	36	0.5 ± 0.1***	0.2 – 1.1	26
Chl *a*	1.1 ± 0.3	0.6 – 1.9	25	1.1 ± 0.3	0.6 – 1.9	25
Chl *b*	1.2 ± 0.3	0.7 – 2.0	23	1.1 ± 0.4	0.3 - 2.2	32
Car	0.9 ± 0.2	0.4 – 1.6	27	1.1 ± 0.2****	0.5 – 2.0	21
TChl/Car	1.3 ± 0.2	0.9 – 2.0	17	1.0 ± 0.2****	0.5 – 1.5	16
E	0.4 ± 0.2	0.1 – 1.2	44	0.4 ± 0.3	01 – 1.1	58
g_s	0.4 ± 0.2	0.1 – 1.0	61	0.3 ± 0.2	0.04 – 0.9	74
P_N	0.9 ± 0.8	0.1 – 5.2	94	0.8 ± 0.6	0.4 – 2.7	75
WUE	2.0 ± 1.2	0.4 – 6.7	78	1.7 ± 1.1	0.4 – 4.8	62
F_0	1.4 ± 0.6	0.7 – 3.2	44	1.0 ± 0.2***	0.6 – 1.6	15
F_m	0.9 ± 0.4	0.4 – 2.4	49	1.0 ± 0.1	0.5 – 1.2	15
F_t	1.2 ± 0.5	0.5 – 3.0	47	1.3 ± 0.3	0.6 – 2.0	22
F_m/F_0	1.0 ± 0.2	0.7 – 1.4	18	1.0 ± 0.2	0.6 – 1.3	15
F_v/F_0	1.0 ± 0.2	0.6 – 1.3	18	1.0 ± 0.2	0.5 – 1.7	23
F_v/F_m	1.0 ± 1.0	0.9 – 1.1	4	1.0 ± 0.1	0.8 – 1.1	5
Φ_{PSII}	1.1 ±0.2	0.7 – 1.8	22	1.0 ±0.2**	0.3 – 1.3	19
NPQ	0.7 ±0.3	0.3 – 1.6	38	0.8 ±0.6	0.4 – 4.6	66
q_N	0.8 ±0.2	0.4 – 1.5	27	0.8 ±0.2	0.4 – 1.4	25
ETR_{160}	1.1 ± 0.3	0.6 – 1.7	22	1.0 ± 0.2**	0.5 – 1.3	18
lk	1.8 ± 1.1	0.2 – 6.2	62	1.3 ± 0.7**	0.3 – 3.4	53
ETR_{max}	1.7 ± 0.3	0.5 – 3.7	49	1.2 ± 0.5***	0.3 – 2.4	44
R_{fd}	0.9 ± 0.2	0.5 – 1.3	19	0.8 ± 0.2	0.5 – 1.5	20

between the STI for the studied attributes was observed under conditions of SD (Table 3). Thus, STI for the biomass correlated positively with STI for the photosynthetic pigments content, F_m, F_t, F_v/F_0, F_v/F_m, and negatively with STI for ETR_{160}. STI for P_N and WUE correlated positively with STI for Φ_{PSII} and ETR_{160}, and negatively with NPQ and q_N. Under SD conditions, STI for Chl *a*, Chl *b*, and TChl/Car positively correlated with STI for F_m, F_t, F_v/F_0, F_v/F_m, and ETR_{max}. STI for F_0, F_m, and F_t negatively correlated with STI for Φ_{PSII}, ETR_{160}, and ETR_{max}. Correlation relationships between STI for Φ_{PSII} and STI for NPQ and q_N were negative, as they were under SAD conditions. Under SD, the profile of correlations between STI for R_{fd} and the one for all the studied parameters was remarkably different. STI for R_{fd} correlated positively with STI for Φ_{PSII}, q_N, lk, ETR_{160}, and ETR_{max}, and negatively with STI for F_0, F_m, and F_t. Both in the first and in the second independent experiment, no significant correlation between STI for R_{fd} and STI for P_N was found.

Under conditions of the climatic chamber *CLF PlantMaster*, the activities of a few enzymes of the water-water cycle were measured, and remarkable positive correlation relationships between STI for the activity of APX, DHAR, GR, and STI for the biomass were identified. STI for all of the four studied activities positively correlated with STI for P_N, and STI for SOD and DHAR positively correlated with STI for Φ_{PSII} and ETR. STI for SOD positively correlated with STI for R_{fd} (Table 4).

Discussion

Stress tolerance index as defined by Fernandez (1993) is considered a good tool to evaluate the genotype tolerance based on the morphometric features and yield components in breeding programs (Naghavi *et al.* 2013, Ali and El-Sadek 2016). STI allows us to quantify changes in a parameter of a specific genotype relatively to the average level of this parameter in the group of genotypes. We chose this index as the most relevant one for the analysis of the photosynthetic parameters tolerance in the set of CS/Syn RILs depending on the stress intensity. Despite the significant variability of STI for most studied parameters, average values of this index allowed us to analyze the main tendencies of changes in the attributes under water

Table 2. Coefficients of correlation between the average STI for shoot biomass and photosynthetic parameters in CS/SynRILs grown under conditions of soil-atmospheric drought. BM − shoot biomass; Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; Car – carotenoids; ETR_{160} –electron transport rate at 160 μmol(photon) m^{-2} s^{-1}; ETR_{max} – maximum electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_t – stationary Chl fluorescence; F_V/F_m – maximum quantum yield of PSII photochemistry; F_V/F_0 – the contribution of the light reactions to primary photochemistry; g_s – stomatal conductance; lk – intensity of illumination, expressing the beginning of PAR saturation; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; q_N – nonphotochemical quenching coefficient; R_{fd} – vitality index; TChl – total chlorophyll; WUE – water-use efficiency (= P_N/E); Φ_{PSII} – effective quantum yield of PSII photochemistry. [a] − significant at $p<0.05$, [b] − at $p<0.01$, [c] – at $p<0.001$, ns − not significant.

	BM	E	g_s	P_N	WUE	Chl *a*	Chl *b*	TChl	Car	TChl/Car	F_0	F_m	F_t	F_v/F_0	F_v/F_m	Φ_{PSII}	NPQ	q_N	ETR_{160}	lk	ETR_{max}
E	–0.25[a]	1																			
g_s	–0.23[a]	0.90[c]	1																		
P_N	ns	0.32[b]	0.46[c]	1																	
WUE	0.25[a]	ns	ns	0.79[c]	1																
Chl *a*	ns	ns	ns	ns	ns	1															
Chl *b*	ns	ns	ns	ns	ns	0.83[c]	1														
TChl	ns	ns	ns	ns	ns	0.96[c]	0.90[c]	1													
Car	ns	ns	ns	ns	ns	0.81[c]	0.67[c]	0.76[c]	1												
TChl/Car	ns	ns	ns	ns	ns	ns	Ns	ns	–0.61[c]	1											
F_0	–0.50[c]	ns	ns	ns	ns	–0.43[c]	–0.39[b]	–0.43[c]	–0.44[c]	0.23[a]	1										
F_m	–0.50[c]	ns	ns	ns	ns	–0.39[b]	–0.36[b]	–0.39[b]	–0.40[c]	ns	0.96[c]	1									
F_t	–0.41[c]	–0.29[a]	–0.23[a]	ns	ns	–0.33[b]	–0.30[b]	–0.35[b]	–0.35[b]	ns	0.92[c]	0.97[c]	1								
F_v/F_0	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.30[b]	0.31[b]	1							
F_v/F_m	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.33[b]	0.34[b]	0.80[c]	1						
Φ_{PSII}	–0.33[b]	ns	ns	ns	ns	ns	ns	–0.23[a]	ns	ns	0.54[c]	0.60[c]	0.58[c]	0.28[a]	0.37[b]	1					
NPQ	ns	0.44[c]	0.34[b]	ns	ns	ns	ns	ns	ns	ns	–0.40[c]	–0.42[c]	–0.52[c]	ns	–0.23[a]	–0.76[c]	1				
q_N	ns	0.40[c]	0.28[a]	ns	ns	0.25[a]	ns	0.28[a]	ns	ns	–0.34[b]	–0.34[b]	–0.50[c]	–0.24[a]	–0.32[b]	–0.66[c]	0.94[c]	1			
ETR_{160}	0.31[b]	ns	ns	ns	ns	ns	ns	ns	ns	ns	–0.36[b]	–0.36[b]	ns	0.29[a]	0.39[b]	0.48[c]	–0.49[c]	–0.51[c]	1		
lk	0.26[a]	ns	ns	0.23[a]	0.26[a]	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.30[b]	0.24[a]	–0.50[c]	–0.51[c]	0.60[c]	1	
ETR_{max}	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.33[b]	0.43[c]	–0.51[c]	–0.54[c]	0.70[c]	0.77[c]	1
R_{fd}	–0.34[b]	0.43[c]	0.28[a]	ns	–0.29[a]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.51[c]	0.54[c]	–0.30[b]	–0.49[c]	–0.33[b]

Table 3. Coefficients of correlation between the average STI for shoot biomass and photosynthetic parameters in CS/Syn RILs grown under conditions of soil drought. BM − shoot biomass; Chl – chlorophyll; Car – carotenoids; ETR_{160} – electron transport rate at 160 μmol(photon) m^{-2} s^{-1}; ETR_{max} – maximum electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_t – stationary Chl fluorescence; F_V/F_m – maximum quantum yield of PSII photochemistry; F_V/F_0 – the contribution of the light reactions to primary photochemistry; g_s – stomatal conductance; lk – intensity of illumination, expressing the beginning of PAR saturation; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; q_N – nonphotochemical quenching coefficient; R_{fd} – vitality index; TChl – total chlorophyll; WUE – water-use efficiency (= P_N/E); Φ_{PSII} – effective quantum yield of PSII photochemistry. [a] − significant at $p<0.05$, [b] − at $p<0.01$, [c] – at $p<0.001$, ns − not significant.

	BM	E	g_s	P_N	WUE	Chl *a*	Chl *b*	TChl	Car	TChl/Car	F_0	F_m	F_t	F_v/F_0	F_v/F_m	Φ_{PSI}	NPQ	q_N	ETR_{160}	lk	ETR_{max}
E	ns	1																			
g_s	ns	0.68[c]	1																		
P_N	ns	0.46[b]	0.26[a]	1																	
WUE	ns	-0.40[c]	-0.29[a]	0.46[c]	1																
Chl *a*	0.29[a]	ns	ns	ns	ns	1															
Chl *b*	0.27[a]	ns	ns	ns	ns	0.82[c]	1														
TChl	0.30[b]	ns	ns	ns	ns	0.97[c]	0.93[c]	1													
Car	0.27[a]	ns	ns	ns	-0.24[c]	0.83[c]	0.57[c]	0.76[c]	1												
TChl/Car	ns	ns	ns	ns	ns	0.44[c]	0.67[c]	0.56[c]	ns	1											
F_0	ns	ns	ns	ns	ns	Ns	Ns	ns	ns	ns	1										
F_m	0.37[b]	ns	ns	ns	ns	0.36[b]	0.36[b]	0.38[b]	ns	0.26[a]	0.47[c]	1									
F_t	0.36[b]	-0.23[a]	ns	ns	ns	0.37[b]	0.30[b]	0.36[b]	0.28[a]	ns	0.51[c]	0.84[c]	1								
F_v/F_0	0.37[b]	-0.25[a]	ns	ns	ns	0.34[b]	0.36[b]	0.36[b]	ns	0.29[a]	-0.39[b]	0.51[c]	0.32[b]	1							
F_v/F_m	0.26[a]	-0.30[b]	ns	ns	ns	0.28[a]	0.29[a]	0.29[b]	ns	0.28[a]	-0.35[b]	0.52[c]	0.32[b]	0.73[c]	1						
Φ_{PSII}	ns	0.25[a]	ns	0.38[b]	0.23[a]	ns	ns	ns	ns	ns	-0.44[c]	-0.41[c]	-0.55[c]	ns	ns	1					
NPQ	ns	ns	ns	–0.27[a]	ns	–0.27[a]	ns	–0.25[a]	–0.32[b]	ns	Ns	0.24[a]	ns	ns	ns	-0.67[c]	1				
q_N	ns	ns	ns	–0.35[b]	–0.26[a]	–0.38[b]	ns	–0.32[b]	–0.41[b]	ns	Ns	ns	ns	ns	ns	-0.58[c]	0.72[c]	1			
ETR_{160}	–0.26[a]	0.25[a]	ns	0.37[b]	0.23[a]	ns	ns	ns	ns	ns	–0.45[c]	–0.46[c]	–0.61[c]	ns	ns	0.96[c]	–0.55[c]	–0.56[c]	1		
lk	ns	0.33[b]	ns	ns	ns	ns	ns	ns	0.29[a]	ns	ns	–0.31[b]	–0.38[b]	ns	ns	0.47[c]	–0.31[b]	–0.28[a]	0.51[c]	1	
ETR_{max}	ns	0.30[b]	ns	ns	ns	0.33[b]	0.26[a]	0.32[b]	0.42[c]	ns	–0.30[b]	–0.23[a]	–0.36[b]	ns	ns	0.66[c]	–0.43[c]	–0.48[c]	0.67[c]	0.72[c]	1
R_{fd}	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	–0.36[b]	–0.27[a]	–0.73[c]	ns	ns	0.50[c]	ns	0.29[a]	0.54[c]	0.27[a]	0.34[b]

Table 4. Correlations between the average STI for the activity of the enzymes of the water-water cycle and STI for biomass and photosynthetic parameters in CS/Syn RILs, grown under soil drought conditions. APX – ascorbate peroxidase; BM − shoot biomass; Chl – chlorophyll; Car – carotenoids; DHAR – dehydroascorbate reductase; ETR_{160} – electron transport rate at 160 μmol (photon) m^{-2} s^{-1}; ETR_{max} – maximum electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_t – stationary Chl fluorescence; F_V/F_m – maximum quantum yield of PSII photochemistry; F_V/F_0 – the contribution of the light reactions to primary photochemistry; GR – glutathione reductase; g_s – stomatal conductance; lk – intensity of illumination, expressing the beginning of PAR saturation; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; q_N – nonphotochemical quenching coefficient; R_{fd} – vitality index; SOD – superoxide dismutase; STI – glutathione reductase; TChl – total chlorophyll; WUE – water-use efficiency (= P_N/E); Φ_{PSII} – effective quantum yield of PSII photochemistry. [a] – significant at $p<0.05$, [b] − at $p<0.01$, [c] – at $p<0.001$, ns − not significant.

STI	SOD	APX	DHAR	GR
BM	ns	0.28[a]	0.23[a]	0.24[a]
E	0.24[a]	ns	ns	–0.20[a]
g_s	ns	ns	ns	ns
P_N	0.34[b]	0.41[c]	0.23[a]	0.32[b]
WUE	ns	ns	ns	0.39[b]
Chl *a*	ns	ns	ns	0.27[a]
Chl *b*	ns	ns	ns	ns
TChl	ns	ns	ns	0.23[a]
Car	ns	ns	ns	ns
TChl/Car	ns	0.26[a]	ns	0.33[b]
F_0	ns	ns	ns	ns
F_M	–0.42[c]	ns	ns	ns
Ft	–0.48[c]	ns	ns	ns
F_v/F_0	–0.31[b]	ns	ns	ns
F_v/F_m	–0.29[a]	ns	ns	ns
Φ_{PSII}	0.52[c]	ns	0.37[b]	ns
NPQ	–0.36[b]	ns	–0.33[b]	–0.23[a]
q_N	–0.30[b]	ns	–0.41[b]	–0.23[a]
ETR 160	0.54[c]	ns	0.37[b]	ns
lk	0.34[b]	ns	ns	ns
ETR_{max}	0.36[b]	ns	0.29[a]	ns
R_{fd}	0.31[b]	ns	ns	ns

stress. Stomatal effects, undoubtedly, played a crucial role in the limitation of photosynthesis in the plant adaptation to water stress, however, the average values of STI for the gas-exchange parameters did not differ under SD and SAD conditions (Table 1). Probably, the differences in the biomass tolerance, characteristic of the studied plants undergoing water stress of various intensity, were conditioned by nonstomatal effects, and, in particular, by the differences in the efficiency of the defense response at the cellular level. We observed considerable differences in the total Car content (Table 1). Although we did not address this question specifically, we can suppose that the increase in the Car content observed under SD conditions was provided both by primary and secondary Car. Positive correlations of STI for Car with STI for F_v/F_0 and F_v/F_m are illustrative of the important role the primary Car play supporting PA functions (Table 3). Along with that, a considerable amount of secondary Car is known to be accumulated in many phototrophic organisms under the influence of unfavourable conditions, including drought (Boussiba 2000). As Solovchenko and Neverov (2017) indicate, secondary Car are very resistant molecules. Once they appear, they remain in the cell for a long time, increasing its tolerance by (*1*) optical shielding of the cell from excessive PAR, (*2*) providing the sink of excessive photosynthates, (*3*) by consumption of O_2 and (*4*) due to a local antioxidant effect. These attributes of the secondary Car are very important for the adaptation observed in our experiments to long-term water stress. However, under more severe conditions combining soil and atmospheric water stress, there was no increase in the Car content, which can probably be explained by failures in the mechanisms of Car synthesis.

Unlike much data concerning decrease in the Chl content in leaves of wheat and other plants (Keyvan 2010, Nikolaeva *et al.* 2010, Chen *et al*. 2015, Li *et al.* 2017, Pour-Aboughadareh *et al*. 2017) under water stress, we observed the increase in Chl *a* and Chl *b* under unfavourable conditions. This can be both related to the increase in the Chl molecule concentration per unit mass of a desiccated leaf and the phenomenon 'stay-green' when PA damage is partially or fully prevented during the leaf-ageing period (Thomas and Howarth 2000). Genetic and physiological reasons for the 'stay-green' effect are diverse; in particular, Rivero *et al*. (2007, 2009) showed that the increased cytokinin production delayed the senescence of transgenic tobacco leaves induced by drought. Presumably, the increased Chl content, which we observed under SD conditions, was 'functional stay-green', as the Chl content tolerance positively correlated with the one of the Chl fluorescence parameters and biomass (Table 3). Under SAD, STI for the biomass did not correlate with STI for Chl, while the correlations between the tolerance of the photosynthetic parameters and the one of the pigment content were negative (Table 2). We believe that this is related to the 'non-functional stay-green' state, when leaves remain green due to the disturbance of Chl catabolism, but the photosynthetic ability is reduced, probably because of the unfavourable modifications of pigment–protein complexes under the influence of water stress (Thomas and Howarth 2000). The genetic background for 'stay-green' is well studied for rice (Jiang *et al*. 2004), wheat (Kumar *et al*. 2010), and another crop plant species (Thomas and Howarth 2000). The ability of CS/Syn RILs to maintain the high Chl content under conditions of water stress presumably has a genetic background. As we observed earlier, both in the parental genotypes of selection variety Chinese Spring, the habitat of which belongs to the southern regions of China, and in the ones of the synthetic hexaploid Synthetic 6x, obtained by means of hybridisation of wild ancestors of wheat species *Triticum dicoccoides* and *Aegilops tauschii* (McFadden and Sears 1946), the Chl content per unit fresh

leaf mass increased under conditions of drought (Osipova *et al.* 2016).

In all the RILs, both under SD and SAD conditions, the ratio F_m/F_0 fluctuated around 4, F_v/F_m fluctuated around 0.8, which meant that PSII was well preserved (Goltsev *et al.* 2016).

Among the parameters of Chl fluorescence, F_v/F_m, F_m/F_0, and F_v/F_0 were the most stable ones, which is consistent with the data provided by M. Zivcak *et al*. (2008, 2013) for winter wheat as well as with the data published by Lichtenthaler *et al*. (2005). We observed a significant increase in STI for F_0 in RILs only under SAD conditions, which was indicative of a relative tolerance of the excitation energy transfer processes in the light-harvesting antenna of PSII in wheat.

In our experiments, the parameters of the fast light curve based on Chl fluorescence were the most sensitive to the intensity of water stress. In particular, they concerned lk and ETR_{max}, their STI tended to increase proportionally to the level of the stress. At the same time, average STI values for NPQ and q_N were less than 1 regardless of growing conditions; as in most RILs (under SAD, 85%; under SD, 75%), NPQ and q_N values were lower under stress conditions. In the experiments carried out by Zivcak *et al*. (2013) with winter wheat leaves (*Triticum aestivum* L.) to different drought conditions, an increase in NPQ took place when the water stress reached the critical level and the stomata were almost fully closed. The threshold level of g_s, where NPQ significantly increased, was about 0.12 mmol m^{-2} s^{-1}. In our experiments, the average value of g_s under SD was 0.041 mol m^{-2} s^{-1} with the range of variation 0.01–0.09 mol m^{-2} s^{-1}; under SAD, it was 0.029 mol m^{-2} s^{-1} with the range of variation 0.01−0.05 mol m^{-2} s^{-1}. Apparently, under conditions of our experiments, most RILs did not reach the threshold level of g_s, which could influence the increase in NPQ. It was as very likely that under water stress, the decrease in nonphotochemical quenching of Chl fluorescence and the increase in ETR_{max} were related to the 'stay-green' phenomenon, as a result of which the cyclic electron transport enhanced around PSII and PSI.

It is to be noted that STI for ETR_{160}, lk, and ETR_{max} positively correlated with STI for R_{fd} under soil-drought conditions, and negatively under soil-atmospheric-drought conditions. As R_{fd} reflected the interaction of the light-dependent photosynthetic reactions with the reactions of the dark phase (Goltsev *et al.* 2016), it was obvious that, under SAD, the imbalance of photosynthetic regulatory mechanisms as well as the enhancement of cyclic electron flow around PSII and PSI took place, which was also highlighted in the experiments performed by Zivcak *et al*. (2013). Under greenhouse conditions, in addition to the soil-atmospheric water stress, there were natural fluctuations in light, which had a considerable influence on the efficiency of photosynthesis and the phenotype of plants (Vialet-Chabrand *et al*. 2017).

The water–water cycle is one of the alternative electron sinks and a powerful mechanism that protects the photosynthetic apparatus from damage by active forms of oxygen under stress conditions, including drought (Foyer and Shigeoka 2011, Ivanov 2014). The positive correlations between STI for the activity of the enzymes of the water–water cycles and STI for biomass, P_N, and Φ_{PSII} (Table 4) indicate the important role of the water–water cycle in the stability of the photosynthetic apparatus in CS/Syn RILs.

Conclusion: Our research identified a high tolerance of PA in leaves from CS/Syn RILs to water stress. The results showed that changes in the studied photosynthetic parameters during the adaptation of RILs to water deficiency depended on the degree of stress; under different conditions, different protective mechanisms are effective. So, the carotenoid response was found only under SD. At the same time, some unusual reactions to water stress were found: the increase in the Chl content and the decrease in the parameters of nonphotochemical fluorescence quenching. The chlorophyll fluorescence parameters, F_v/F_m, F_m/F_0, and F_v/F_0, which are often used to assess the physiological state of plants (Goltsev *et al*. 2016), were constant in our experiments and did not reflect changes in the plant status. The parameters of the fast light curve based on chlorophyll fluorescence, lk and ETR_{max}, were the most sensitive to water supply conditions; these parameters were increasing proportionally to the stress level. In general, it seems that the cyclic transport of electrons around PSII and PSI, as well as the electron sink into the water–water cycle, were the main protective mechanisms of the photosynthetic apparatus of wheat. This conclusion is in line with the findings of Zivcak *et al*. (2013).

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