

CHANGES IN CO₂ ASSIMILATION, TRANSPIRATION AND STOMATAL RESISTANCE OF DIFFERENT WHEAT CULTIVARS EXPERIENCING DROUGHT UNDER FIELD CONDITIONS

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Summary. The response of the photosynthetic apparatus of different wheat cultivars (cvs) of *Triticum aestivum* L. to drought was investigated. The cvs. were sowed in a special “desiccation greenhouse”, i.e. field area covered by polyethylene in order to prevent plant watering by rain. Photosynthetic CO₂ uptake, stomatal resistance and transpiration were measured on the flag leaf with a portable photosynthetic system LI 6000 (Li-Cor, USA). It was found that mild water deficit (WD) decreased the rate of photosynthesis to a different extent depending on drought tolerance of the cvs. With the further increase of drought, the reduction of photosynthetic rate increased and the wheat cvs. demonstrated rather different drought tolerance compared to mild WD. In most cases no direct correlation between the extent of reduction in photosynthetic rate, transpiration and stomatal resistance was found. On the basis of the data obtained it was suggested that even under mild WD the stomatal resistance was not always the main factor limiting photosynthesis in droughted plants. Especially under severe WD the photosynthetic CO₂ uptake depended not only on transpiration rate and stomatal resistance. Obviously, under such conditions non-stomatal factors, e.g. chloroplast capacity to fix CO₂ limited to a greater extent the rate of photosynthesis. The differences in drought tolerance of wheat cvs. in dependence of the severity of WD, reported here may be related to the extent of “physiological window” (Burke, 1990).

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On the basis of the data characterizing net photosynthetic rate and WUE the cultivars were divided in three groups.

Key words: wheat, photosynthesis, stomatal resistance, transpiration, drought tolerance

Abbreviations: Chl – chlorophyll, C_i – intercellular CO₂, cv(s) – cultivar(s), RuBP – ribulose-1,5-bisphosphate, Rubisco – ribulose-1,5-phosphate carboxylase-oxygenase, WD – water deficit, WUE – water use efficiency

Introduction.

In the field plants are often exposed to environmental stresses, especially drought, high light, high and low temperature. As Blum (1996) noted, drought is a multidimensional stress affecting plants at various levels of their organization. At the whole plant and crop levels, the plant response to drought is complex because it reflects the integration of stress effects and responses at all underlying levels of organization over space and time. At the whole plant level, the effect of stress is usually perceived as a decrease in photosynthesis and growth (Cornic and Massacci, 1996; Mwanamwenge et al., 1999). The rate of CO₂ assimilation in the leaves was reduced at moderate water deficits (WD) (Kaiser, 1987; Sharkey and Seeman, 1989; Lawlor and Uprety, 1993; Yordanov et al., 1997) or even before leaf water status was changed in response to a drop in air humidity (Bunce, 1981), or in soil water potential (Gollan et al., 1986; Socias et al. 1997). Measurements of gas exchange and chlorophyll (Chl) fluorescence in bean plants during water and high temperature stresses showed that water deficit (17–20%) caused a significant decrease in the rate of CO₂ uptake and O₂ evolution (Yordanov et al., 1997, 1998).

Several lines of evidence indicate that a decrease in photosynthesis due to WD has been attributed to both stomatal and non-stomatal limitations (Graan and Boyer, 1990; Ort et al., 1994; Shangguan et al., 1999). Stomatal closure is the first line of defense against desiccation, since it is much quicker than e.g. changes in roots growth, leaf area, chloroplast ultrastructure and pigment proteins. In many cases the more drought tolerant species control stomatal function to allow some carbon fixation at stress, thus improving WUE or open stomata rapidly when WD is relieved. The relative part of stomatal limitation of photosynthesis depends on severity of WD. At mild stress it is primal event, which is then followed by adequate changes of photosynthetic reactions (Cornic and Briantais, 1991). At long-term WD the non stomatal limitation predominates.

Except by increased diffusive resistance (Faver et al., 1996; Herpich and Peckmann, 1997), photosynthesis may also be controlled by the chloroplast's capacity to fix CO₂. The non stomatal limitation of photosynthesis may be attributed to reduced

carboxylation efficiency (Wise et al., 1991), reduced ribulose-1,5-bisphosphate (RuBP) regeneration (Gimenez et al., 1992; Tezara and Lawlor, 1995), or to a reduced amount of functional Rubisco (Kanechi et al., 1995).

There are experimental data showing that each stress monitored under controlled conditions in the laboratory might be quite different from the plant response in the field where several factors interact. Therefore, the aim of this investigation was to compare the drought tolerance of 23 cultivars (cvs), growing in the field with that observed in laboratory conditions at controlled light and temperature. As a criterion we used the functional activity of photosynthetic apparatus (PSA), evaluated by different methods and parameters.

Materials and Methods

The experiments were carried out at the Institute of wheat and sunflower in Dobrich. The following 23 cultivars (cvs) were studied: Aglika, Albena, Bezostaya 1, Charodeika, Elitsa, Flamura 80, Yantar, Kristal, Laska, Murgavets, Mustang, Prelom, Pliska, Pobeda, Preslav, Priaspa, Sadovo, Slavianka 196, Spartanka, Svilena, Todora, Vratsa and Zora.

The wheat cvs were sowed at the end of October in four replications (0.5 m² each) in a special “desiccator greenhouse” in lines of one meter long and 20 cm between the lines. Each replica contained 200 plants. The “desiccator greenhouse” area was covered by polyethylene in order to prevent plants watering by rain. The penetration of water from control plots into those of droughted wheat plots was prevented by drainage. The seedlings germinated in the second half of October and spent the winter good developed in the phase tillering, without injuries caused by low temperatures. During the whole vegetation the plants received 81.8 L.m⁻² water. The last watering was at the end of the first decade of March. The symptoms of WD were observed in the first decade of April when all plants were in the phase onset of shooting. The WD was about 10–15% after 30 days of drought (mild water stress) and varied between 25 and 30% after 40 days of drought (strong water stress) when measurements were carried out.

Photosynthetic CO₂ uptake, stomatal resistance and transpiration were measured with a portable photosynthetic system LI-6000 (Li-Cor, USA) at a leaf temperature in the range of 32–35°C and natural photon flux density (PFD) 1000–1200 μmol.m⁻².s⁻¹. The CO₂ concentration in the leaf chamber was about 350 μl.l⁻¹ ppm. Water deficit of leaves was calculated according to the following formula:

$$\text{WD [\%]} = \frac{(\text{water content at full turgescence} - \text{real water content})}{(\text{water content at full turgescence})} \times 100$$

Data are the means of measurements of 6-8 plants.

Statistics: Values obtained were expressed as mean \pm SE from measurements of five plants of each variant.

Results

Changes in photosynthetic CO₂ uptake. The rate of photosynthesis of control and experienced not very severe WD plants of different cultivars (cvs) is shown in Table 1. The rate of photosynthesis in the control well watered mature flag leaf varied in the range of 0.961 mg.m⁻².s⁻¹ (cv Kristal) to 0.496 mg.m⁻².s⁻¹ (cv.Murgavets). Three of the cvs – Laska, Mustang and Pliska photosynthesised at rates over 0.800 mg.m⁻².s⁻¹, whereas Sadovo, Albena, Aglika, Vratsa, Besostaya – over 0.7 mg.m⁻².s⁻¹. Most of the cvs – Slavianka, Priaspa, Preslav, Pobeda, Yantar, Prelom, Zora, Flamura, Elitsa – photosynthesised at rates over 0.6 mg.m⁻².s⁻¹.

Ten days later, the rate of photosynthesis of control flag leaf was over 0.9 mg CO₂.m⁻².s⁻¹ in Elitsa and Vratsa cvs, over 0.8 mg CO₂.m⁻².s⁻¹ in cvs Yantar, Elitsa and Laska, over 0.7 mg CO₂.m⁻².s⁻¹ in Charodeika, Svilena, Pliska, Todora, Prelom, Sadovo and Aglika. A again, the rate of photosynthesis of control flag leaf in most wheat cvs – Priaspa, Zora, Pobeda, Slavianka, Albena, Mustang, Spartanka) was over 0.6 mg CO₂.m⁻².s⁻¹. Only in 3 cvs – Besostaya, Kristal and Murgavets it was below 0.6 mg CO₂.m⁻².s⁻¹.

Our results showed also that relatively mild WD caused a decrease in the rate of photosynthesis to a different extent, depending on the drought tolerance of cultivars (Table 1). The reduction of CO₂ uptake varied from 17% (cv Todora) to 85% (cv Pliska). The most drought tolerant were the plants of cv. Todora. The photosynthetic rate of their flag leaf was reduced only by 17% compared to control. In the rest 9 cvs – Priaspa, Flamura, Zora, Svilena, Laska, Slavianka 196, Preslav, Mustang and Vratsa, the reduction of CO₂ uptake caused by WD was below 50%. An inhibition of photosynthetic rate over 50 to 60% was observed in cvs Murgavets, Elitsa, Albena, Spartanka, Sadovo, Aglika, Pobeda and Charodeika. The strongest inhibition of photosynthesis of the flag leaf was observed in plants of cvs Yantar, Prelom, Kristal and Pliska.

With the further increase of drought the inhibition of photosynthetic rate increased (Table 2) and was in the range of 37% (cv. Zora) to 91% (cv.Pliska). It is also interesting to mention that under severe drought stress the cultivars demonstrated rather different drought tolerance. Some cvs showing the highest degree of resistance at mild WD, exhibit then the highest inhibition of photosynthesis. For example, under mild drought cv. Todora showed a minimal inhibition (17%), but under severe drought the inhibition was among the highest ones (88%).

Hence, under strong drought conditions some of cvs. change their position with respect to their drought tolerance (compare Table 1 and Table 2).

Table 1. Influence of mild water deficit (30 days drought) on gas exchange parameters in wheat cultivars. The measurements were carried out on the flag leaf.

Cultivar	Net photosynthetic rate mg CO ₂ ·m ⁻² ·s ⁻¹		% of control	Transpiration mg H ₂ O·m ⁻² ·s ⁻¹		Stomatal resistance s·cm ⁻¹		WUE mg CO ₂ /g H ₂ O		A/g _s mg CO ₂ ·m ⁻³	
	Control	Drought		Control	Drought	Control	Drought	Control	Drought	Control	Drought
Todora	0.816±0.136	0.677±0.250	83.0	439±32	480±58	0.20±0.09	0.24±0.13	1.86	1.41	16.2	16.2
Priaspia	0.696±0.179	0.525±0.120	75.5	412±68	387±55	0.24±0.08	0.35±0.08	1.69	1.36	16.8	18.4
Flamura 80	0.618±0.174	0.450±0.112	72.9	386±46	350±32	0.33±0.07	0.46±0.09	1.60	1.28	20.7	20.7
Zora	0.637±0.091	0.441±0.046	69.2	351±44	364±75	0.31±0.07	0.41±0.06	1.81	1.21	19.7	18.1
Svilena	0.558±0.104	0.383±0.138	68.7	321±36	319±33	0.34±0.01	0.44±0.06	1.74	1.20	18.9	16.9
Laska	0.865±0.130	0.568±0.146	65.7	465±31	512±16	0.20±0.07	0.25±0.11	1.86	1.10	17.7	14.1
Slavianka 196	0.698±0.064	0.458±0.150	65.5	351±36	309±47	0.42±0.07	0.72±0.10	1.99	1.48	29.7	32.9
Preoslav	0.659±0.107	0.394±0.218	59.8	358±57	384±71	0.29±0.01	0.42±0.05	1.84	1.03	19.4	16.5
Mustang	0.855±0.018	0.482±0.076	56.4	401±23	383±13	0.21±0.06	0.37±0.11	2.13	1.26	18.2	17.7
Vratsa	0.740±0.185	0.387±0.096	52.2	347±83	363±43	0.49±0.29	0.38±0.16	2.13	1.07	36.6	14.7
Murgavets	0.496±0.165	0.244±0.057	49.1	358±16	318±13	0.28±0.10	0.42±0.07	1.39	0.77	14.1	10.2
Elitsa	0.618±0.030	0.284±0.022	46.1	451±28	463±31	0.20±0.06	0.27±0.14	1.37	0.61	12.1	7.6
Albena	0.776±0.084	0.339±0.206	43.7	421±24	378±62	0.20±0.20	0.44±0.09	1.84	0.90	15.2	14.9
Spartanka	0.586±0.079	0.247±0.043	42.2	340±24	314±34	0.39±0.07	0.60±0.12	1.72	0.79	22.8	14.8
Sadovo	0.782±0.109	0.326±0.082	41.7	375±25	342±38	0.30±0.07	0.50±0.12	2.09	0.95	23.5	16.3
Aglika	0.741±0.072	0.308±0.138	41.5	431±20	453±47	0.25±0.10	0.32±0.16	1.72	0.68	18.8	9.8
Pobeda	0.658±0.137	0.241±0.180	36.6	379±41	448±75	0.30±0.11	0.26±0.13	1.74	0.84	19.8	6.1
Bezostaiia 1	0.717±0.096	0.262±0.099	36.5	341±35	366±59	0.33±0.16	0.36±0.17	2.10	0.72	23.5	9.4
Charodeika	0.710±0.156	0.250±0.115	35.2	405±83	328±41	0.36±0.22	0.46±0.19	1.75	0.76	25.8	11.6
Yantar	0.657±0.112	0.220±0.168	33.5	410±28	367±30	0.26±0.05	0.43±0.12	1.60	0.60	17.0	9.5
Prelom	0.649±0.010	0.209±0.035	32.2	319±43	263±26	0.42±0.16	0.78±0.24	2.03	0.79	27.3	16.3
Kristal	0.961±0.172	0.264±0.112	27.5	479±29	459±46	0.16±0.06	0.35±0.15	2.01	0.58	15.4	9.2
Pliska	0.806±0.130	0.124±0.095	15.4	331±20	286±26	0.39±0.06	0.71±0.10	2.44	0.43	31.6	8.9

Table 2. Influence of severe water deficit (40 days drought) on gas exchange parameters in wheat cultivars. The measurements were carried out on the flag leaf.

Cultivar	Net photosynthetic rate mg CO ₂ ·m ⁻² ·s ⁻¹		% of control		Transpiration mg H ₂ O·m ⁻² ·s ⁻¹		Stomatal resistance s·cm ⁻¹		WUE mg CO ₂ /g H ₂ O		A/g _s mg CO ₂ ·m ⁻³	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Zora	0.672±0.071	0.424±0.087	63.1	307±88	0.45±0.16	0.80±0.27	1.92	1.38	30.1	34.1		
Albena	0.649±0.038	0.336±0.120	51.8	337±54	0.26±0.06	0.48±0.05	1.75	1.00	16.8	16.2		
Spartanka	0.626±0.089	0.319±0.130	51.0	355±41	0.36±0.06	0.50±0.06	1.68	0.90	22.4	16.0		
Svilena	0.751±0.160	0.383±0.076	51.0	309±57	0.44±0.11	1.12±0.64	2.43	1.39	33.2	42.7		
Murgavets	0.525±0.066	0.219±0.145	41.8	330±27	0.34±0.02	0.74±0.15	1.59	0.75	17.6	16.3		
Elitsa	0.971±0.130	0.392±0.133	40.4	372±33	0.27±0.02	0.46±0.15	2.61	1.10	25.9	18.1		
Vratsa	0.912±0.087	0.358±0.139	39.3	357±46	0.37±0.07	0.62±0.11	2.55	1.19	34.2	22.2		
Slavianka 196	0.656±0.064	0.257±0.110	39.1	383±30	0.34±0.03	0.73±0.05	1.71	0.88	22.0	18.6		
Charodeika	0.772±0.129	0.293±0.078	37.9	335±27	0.43±0.06	0.71±0.09	2.30	0.98	33.1	20.9		
Sadovo	0.728±0.123	0.272±0.096	37.4	324±43	0.43±0.04	0.83±0.10	2.25	1.00	31.1	22.5		
Yantar	0.859±0.174	0.290±0.190	33.7	344±19	0.40±0.02	0.89±0.11	2.50	1.13	34.0	25.7		
Priaspas	0.682±0.107	0.205±0.054	30.1	343±59	0.34±0.07	0.66±0.19	1.99	0.67	23.5	13.6		
Laska	0.804±0.226	0.235±0.134	29.2	377±62	0.29±0.08	0.44±0.13	2.13	0.60	23.2	10.4		
Bezostaia 1	0.585±0.100	0.163±0.091	27.8	382±8	0.30±0.04	0.52±0.07	1.53	0.48	17.3	8.4		
Flamura 80	0.824±0.145	0.218±0.053	26.5	442±38	0.27±0.05	0.55±0.06	1.86	0.60	22.5	12.0		
Prelom	0.735±0.086	0.153±0.062	20.8	312±30	0.46±0.06	1.01±0.08	2.36	0.68	33.8	15.5		
Mustang	0.626±0.046	0.115±0.047	18.3	362±26	0.33±0.04	0.76±0.10	1.73	0.43	20.4	8.7		
Preslav	0.747±0.194	0.106±0.049	14.1	362±66	0.35±0.09	0.86±0.35	2.06	0.37	26.2	9.1		
Todora	0.739±0.148	0.090±0.113	12.2	369±62	0.30±0.08	0.78±0.25	2.00	0.31	22.2	7.0		
Kristal	0.554±0.002	0.063±0.091	11.4	318±44	0.41±0.06	0.55±0.02	1.74	0.20	22.8	3.4		
Aglika	0.725±0.052	0.071±0.082	9.9	322±30	0.46±0.07	0.66±0.26	2.25	0.22	33.2	4.7		
Pobeda	0.663±0.086	0.065±0.165	9.8	383±40	0.31±0.08	0.70±0.30	1.73	0.20	20.5	4.5		
Pliska	0.743±0.054	0.062±0.054	8.4	415±52	0.30±0.06	0.67±0.05	1.79	0.20	22.4	4.2		

It is interesting to note that from 23 cvs investigated the photosynthetic rate of 11 cvs endured mild WD was inhibited less than 50% compared with the respective controls (Table 1). After very strong drought, however, only four of them – Mustang, Elitsa, Vratsa and Charodeika were included in the first group of 11 cvs (Table 2). At the same time, some of cvs showing higher sensitivity to mild WD demonstrated higher drought tolerance under severe drought (cvs Albena, Spartanka, Murgavets, Elitsa, Charodeika, Sadovo and Yantar). Under strong WD only 3 of the investigated cvs – Zora, Albena and Spartanka – photosynthesised at rates more than 50% of the respective controls (100%). In other 5 cvs: Murgavets, Elitsa, Vratsa, Slavianka 196 and Charodeika, under the same conditions, CO₂ uptake was reduced about 3-fold. In 7 cvs: Mustang, Preslav, Todora, Kristal, Aglika, Pobeda and Pliska the inhibition of the rate of CO₂ uptake was more than 80%.

The comparison of the extent of the the photosynthetic rate reduction caused by mild WD with the transpiration rate showed that, in most cases, there was no direct correlation between these two parameters. For example, in some cases, when the transpiration rate in droughted plants was higher (109.5%) than in the respective control, the reduction in photosynthesis was only 17% (cv. Todora), while at similar or higher transpiration rate the reduction in photosynthesis was two- or more fold higher (e.g. in cv Pobeda, where the transpiration rate was 118%, and the inhibition of photosynthesis was 63%). Furthermore, there was no correlation between the reduction of transpiration rates and the rates of photosynthesis in plants experienced WD. At the same reduction of transpiration rate (with about 10% compared with respective control), the rate of photosynthesis decreased by 27% in cv. Flamura, but by 53% in cv. Sadovo. In other cases however, when the transpiration rate was close to the control, the rate of photosynthesis was inhibited to a different extent (compare cvs Kristal and Priaspa). In most of the droughted cvs the stomatal resistance increase did not exceed the respective control value (100%). The only exception were cvs Albena and Kristal – 220% and 218% respectively.

There was no good correlation between increased stomatal resistance in droughted flag leaf and decreased photosynthesis. In two cvs with different stomatal resistance (cvs Laska 0.25 cm.s⁻¹, 25% higher than the respective control; and Slavianka 196 – 0.72, 71% higher then the control) the inhibition of photosynthesis was 34%. On the other hand, the inhibition of photosynthesis in cvs Albena and Kristal with identical stomatal resistance (220 and 219%) was 56 and 72%, respectively.

On the basis of the data obtained, it can be suggested that under mild WD the stomatal resistance was the major factor limiting the rate of photosynthesis in droughted plants. It can also be concluded that even under moderate WD the wheat cvs investigated manifested different drought tolerance. Under severe WD, the transpiration rate of droughted plants in most cvs was changed in the range of 70 to 90% (Table 2). Only in cvs Laska and Aglika it was a little higher compared to the controls.

Again, there was not a direct correlation between the transpiration rate and the reduction of photosynthesis. For example, at equal rates of transpiration (87.7% of the control) the rate of photosynthetic CO₂ uptake in cvs Zora and Besostaya was 63.8% and 27.8% respectively. Besides, the drought tolerance of some cultivars was better expressed under strong WD.

The results on the correlation between photosynthetic rate and the stomatal resistance of plants experienced strong WD showed similar tendency to above mentioned (compare Table 1 and 2). In plants of cv. Svilena, flag leaf of which had stomatal resistance 1.12 cm.s⁻¹ (254,5%, control 100%) photosynthetic rate was inhibited by 41%. The same extent of inhibition was found in the flag leaf of cv. Spartanka plants which stomatal resistance was approximately two fold lower (131,6 against 254,5%).

These results support our conclusion that under strong drought non-stomatal factors limit to a greater extent the rate of photosynthesis. At severe stress photosynthesis might be controlled by chloroplast's capacity to fix CO₂ rather than by a diffusive resistance increase (Faver et al., 1996; Herppich and Peckmann, 1997). Severe WD may also affect photosynthetic capacity *per se* and therefore, alter both the C_i/C_a (= intercellular/ambient air CO₂) ratio and stomatal conductance, g_s (Pereira and Chaves, 1993).

It was also interesting to compare the photosynthesis/transpiration ratio, as a measure of water use efficiency (WUE) (Table 1 and 2). It can be seen that in all cvs at both mild and severe WD the WUE was higher in the control plants. The data showed also that, as a rule, at the second measurement, when the flag leaf was 10 days older, the WUE in control plants was higher compared with that observed 10 days earlier with the exception of 4 cvs - Slavianka, Mustang, Kristal and Pliska – where the WUE was a little lower. The highest level of WD led in most cvs to a strong reduction (from 2 to 4-fold) of WUE (cvs Todora, Flamura, Preslav). In other cvs (Zora, Svilena, Vratsa, Sadovo, Murgavets, Spartanka) the values for WUE were equal at both measurements. The drought tolerance is caused to a great extent by the capability of plants to regulate their water relations. In drought tolerant plants the WUE efficiency was higher, unlike the sensitive plants characterized by a lower WUE value (see Table 2). There are a number of possibilities which can help the plants to retain their photosynthetic rate near to normal even under strong soil water deficit. For example, the development of rich and deep root system during long-term drought enhances synthesis of osmolytes improving the water-holding (*retaining*) ability of cells and tissues and the regulation of water relations (Yancey 1994).

The leaf intrinsic gas exchange efficiency (A/g_s) i.e. carbon assimilation rate/stomatal conductance is presented in Table 1. In plants of all cvs, experienced mild drought the A/g_s ratio was either equal or lower as compare to the respective controls. In some cases (cvs Vratsa, Elitsa, Aglika, Pobeda, Besostaya, Pliska) it was two or more fold lower than in their respective controls. Only in two cvs this ratio was a little higher in droughted plants (Table 2).

Similar results concerning A/g_s ratio were found also in plants experienced strong water deficit (Table 2). Under these conditions this ratio was higher only in cvs Zora and Svilena. In all other cvs it was lower than controls. As a rule, this reduction paralleled with the inhibition of photosynthetic rate, caused by WD. These data did not support the results of Osorio et al. (1998), obtained with rooted cuttings of three *Eucalyptus globulus* clones. In their experiments the A/g_s ratio values increased under water stress.

Discussion

Water deficit reduces transpiration rate, stomatal conductance, net CO_2 uptake and growth of crop plants (Scheuermann et al., 1991). During the period of WD, water potential, and relative water content (RWC) decreased with an associated decrease of Rubisco activity, Chl and protein content (Castrilo and Trujillo, 1994). These authors have found a significant correlation among the components of leaf water status and the measured photosynthetic parameters.

Our results showed that under normal water supply the cvs. investigated were characterized by rather different rates of photosynthesis. As drought developed, net CO_2 fixation rate decreased in all cultivars, although not to the same extent, due to decreasing stomatal conductance and transpiration rate. The decrease of photosynthesis caused by WD has been attributed to both stomatal (restricted CO_2 availability) and non-stomatal limitations (Ort et al., 1994, Srivastava and Strasser, 1997, Shangguan, 1999). Stomatal closure was the most prominent determinant for the increased transpiration efficiency observed in plants experienced not very strong WD (Osorio et al., 1998). In addition, our results support their observations that transpiration efficiency did not differ significantly between two levels of soil WD and this fact emphasized the remarkable capability of acclimation of the species to decreased soil water availability. There are also observations that in response to soil drying stomatal conductance is reduced to 50% before any substantial changes in water potential are detected. Socias et al. (1997) showed that the decline in the rate of photosynthetic CO_2 uptake in the first week of drought was less pronounced compared with stomatal conductance, thus increasing WUE.

Cornic and Briantais (1991) found that stomatal conductance in three bean cultivars declined before RWC was affected. Although stomatal closure generally occurs when plants are exposed to drought, in some cases e.g. severe stress, photosynthesis may be controlled by the chloroplast capacity to fix CO_2 (non-stomatal limitation) rather than by the increased diffusive resistance (Faverat et al. 1996, Herppich and Peekmann, 1997). It has been shown that non-stomatal limitation of photosynthesis in sunflower leaves is attributed to reduced carboxylation efficiency (Wise et al., 1991), reduced RuBP regeneration (Gimenez et al. 1992; Tezara and Lawlor, 1995), or to a

reduced amount of functional RuBPCase (Kanechi et al., 1995). According to Lal et al. (1996) there is no evidence that the capacity for RuBP regeneration becomes a limiting factor during drought. It is noteworthy to mention that the inhibition of photosynthesis by drought is a complex phenomenon, since it can be caused by different limitations, stomatal closure (stomatal conductance) and transpiration being only part of them. It was shown that maximal Rubisco activity decreased slightly at moderate WD and sharply at severe drought. The decrease at moderate WD seems to be due to a decrease of Rubisco protein in leaves while at severe drought to a decrease in both Rubisco protein and Rubisco specific activity (Marques et al. 1995). The reduction in the rate of reactions catalysed by Rubisco could be caused either by changes in the availability of CO₂ at chloroplast level or the availability of RuBP, or loss of Rubisco capacity (Lal et al., 1996). Their results indicate that the primary mechanism for inhibition of photosynthesis under both mild and severe WD is a reduced CO₂ supply to Rubisco (under stress the intercellular CO₂ decreases). The ratio of RuBP pool to substrate binding site on Rubisco remains reasonably constant during drought. There are also experimental data showing that Rubisco capacity is not the major limitation during stress (Sharkey, 1987; Sharkey and Seeman, 1989). Lal et al. (1996) showed that in *Vicia faba* photosynthesis began to decrease 2 days after withholding of water, whereas RuBPCase activity did not decrease after 7 days. In experiments of Sharkey (1987), Sharkey and Seeman (1989) Rubisco content and activity were not affected by mild WD. By contrast, according to Vu et al. (1998) both a high CO₂ concentration and severe drought decreased the activity and content of Rubisco.

Lal et al. (1996) adduced some evidence that a decrease in CO₂ supply to Rubisco is predominantly responsible for a decrease in photosynthesis during drought. Indeed, the degree of control by Rubisco increases when CO₂ itself is a major limiting factor but this does not negate the fact that CO₂ itself is the primary limitation and not Rubisco content. Under severe WD the supply of CO₂ to Rubisco may be limited not only by stomatal closure but also by stomatal patchiness and tissue shrinkage diminishing the intracellular air space.

There are enough data showing the existence of adaptive mechanism(s) of land plants to protect themselves against different kinds of stress – light, high temperature, WD (Havaux, 1992; Srivastava and Strasser, 1996). Bajji et al. (2000) have noted that in durum wheat the degree of resistance to drought at the plant level depends, at least in part, on the existence of a mechanism operating at the cellular level. In support of this assumption the authors point out the correlation between performance of cvs under drought and the response of callus culture to PEG-induced drought.

It is necessary to note that the antagonism between stress factors plays a very important role in the adaptation process. It was been found that WD antagonizes the effect of heat stress (Havaux, 1992; Srivastava and Strasser, 1996). Preheated adaptation changes the conformation (lipid-protein interaction) of PS2 which somehow results in protection of PS2 against strong light and partially against drought. Srivas-

tava and Strasser (1996) reported that moderate treatment by low light or moderate temperature and moderate WD induced an extenuation of the buffer capacity of plants against stress, e.g. light, heat, drought. Hence, the data discussed above point to the complexity of photosynthetic responses to environmental stresses, the antagonistic effect of given stress being markedly modulated by another environmental factor(s). In contrast to the synergistic effect, high light and high temperature pretreatment ($30^{\circ}\text{C} + 30 \text{ W.m}^{-2}$, 30 min) results in protection of the photosynthetic mechanism exposed to high light.

Metabolic acclimation via the accumulation of compatible solutes is also regarded as a basic strategy for the protection and survival of plants in extreme environment. The synthesis of betaine in chloroplasts can enhance protection to several types of stress (Sakamoto and Murata, 2000). The major role of metabolites like betaine, sugar alcohols, amino acids and their derivatives is to serve as organic osmolytes with compatible properties at high concentrations. Such osmolytes increase the ability of cells to retain water without disturbing normal cellular functions (Yancey, 1994; Yancey et al., 1982).

Differences between species can also be due to the operation of additional mechanisms functioning in some cultivars but not in others. According to Munne-Bosch and Alegre (2000) the enhanced formation of zeaxanthin in high light and the increased levels of carotenoids per unit of Chl, observed in WD plants, may help to avoid photoinhibitory damage to the photosynthetic apparatus.

Conclusion

Many studies have illustrated the complexity of the photosynthetic responses to environmental stresses with the given stress being markedly modified by other environmental factors. Mild treatment with low light or moderate temperature and moderate water loss induces an extension of the buffer capacity of plants against stress. Out of several mechanisms, the self regulation is the one to integrate the complex interactions and to refine regulations. The results presented in this work suggest that the degree of tolerance to WD of different wheat cultivars is the result of their different plasticity and ability to short and long-term acclimation and may be connected with the extent of the “physiological window” (Burke, 1990) which defines the threshold of drought tolerance. Our results demonstrate also that the drought tolerance observed under laboratory conditions does not necessarily correlate with plant response under field conditions. These data are in support of the conclusion of Srivastava and Strasser (1997) that the interaction between different stress factors suggests that each stress monitored under controlled environmental conditions in the laboratory, where the effects of a defined stress are studied in “one factor - one response” test might be quite different from the plant response in the field where several factors usually change simul-

taneously and interact. On the basis of our data characterizing the rate of photosynthesis and WUE, the cvs investigated can be divided in three groups: (1) drought tolerant – with representatives cvs Zora, Svilena, Albena, Vratsa, Elitsa, Spartanka; (2) drought sensitive – cvs Pliska, Pobeda, Kristal, Aglika, Todora and (3) less sensitive – cvs Preslav, Prelom, Bezostaya.

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