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Drought-Induced Changes in Photosynthetic Membranes of Two Wheat (*Triticum aestivum* L.) Cultivars

I. M. Huseynova*, S. Y. Suleymanov, S. M. Rustamova, and J. A. Aliyev

Institute of Botany, National Academy of Sciences, Patamdar Shosse 40, AZ 1073 Baku, Azerbaijan; fax: (99412) 497-5045; (99412) 438-1164; E-mail: aliyev-j@botany-az.org

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Abstract—Two wheat (*Triticum aestivum* L.) cultivars contrasting in architectonics and differing in drought resistance, Azamatli-95 (short stature, vertically oriented small leaves, drought-tolerant) and Giymatli-2/17 (short stature, broad and drooping leaves, drought-sensitive), were studied. It was found that the content of CP I (115 kDa) and 63-kDa apoprotein P700 and also LHC II polypeptides increases slightly in the drought-resistant cv. Azamatli-95 under extreme water supply limitation, while their content decreases in drought-sensitive cv. Giymatli-2/17. The intensity of synthesis of α- and β-subunits of CF_1 (55 and 53.5 kDa) and 33-30.5 kDa proteins also decreases in the sensitive genotype. The intensity of short wavelength peaks at 687 and 695 nm sharply increases in the fluorescence spectra (77K) of chloroplasts from Giymatli-2/17 under water deficiency, and there is a stimulation of the ratio of fluorescence band intensity F687/F740. After exposure to drought, cv. Giymatli-2/17 shows a larger reduction in the actual PS II photochemical efficiency of chloroplasts than cv. Azamatli-95.

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Under natural conditions plants are frequently subjected to external stresses that limit their growth, crop productivity, and species distribution. Abiotic stress elicits a complex of responses beginning with stress perception, which initiates a signal transduction pathway(s) and is manifested in changes at the cellular, physiological, and developmental levels [1]. The set of responses observed depends upon severity and duration of the stress, plant genotype, developmental stage, and environmental factors providing the stress. A better understanding of the mechanisms that enable plants to adapt to stress and maintain their growth and productivity during stress periods may be critical for the development of new strategies and tools to enhance stress tolerance via genetic manipulations [2].

Wheat is one of the widely cultivated crops in Azerbaijan, where drought is the main abiotic stress limiting its grain yield [3, 4].

Abbreviations: Chl, chlorophyll; CP 43 and CP 47, core antennas of PS II; DCPIP, 2,6-dichlorophenol-indophenol; LHC, light-harvesting complex; LHC I(II), light-harvesting complex of PS I(II); LPO, lipid peroxidation; MV, methyl viologen; PS I(II), photosystem I(II); ROS, reactive oxygen species; RWC, relative water content.

* To whom correspondence should be addressed.

Water stress, like most environmental constraints, generates reactive oxygen species (ROS) within chloroplasts due to excess excitation energy in relation to limited CO_2 fixation capacity [5, 6]. The reaction centers of photosystem I (PS I) and II (PS II) in chloroplast thylakoids are the major sites of generation of ROS. Prompt scavenging of the ROS produced in thylakoids via antioxidative systems occurs under normal conditions and is indispensable to protect the target molecules in thylakoids and stroma [7]. All ROS are involved in normal cell metabolism, participating in synthesis of certain compounds, and destruction of damaged membrane structures. Production of ROS increases dramatically during stress and causes oxidative burst; ROS damage cells and lead to cell death [8]. They damage protein structures and inactivate them and lead to lipid peroxidation and DNA mutations. Lipid peroxidation (LPO) occurring under the effect of superoxide radicals results in unsaturation of thylakoid lipids and decrease in the fluidity of membranes [9]. To prevent ROS formation, plants have evolved mechanisms such as heat dissipation of excess energy through carotenoids.

Functionality of thylakoid membranes requires mobility of protein components and redox carriers. Photosynthetic electron transfer reactions that take place

in thylakoid membranes are remarkably resistant to dehydration. Membrane proteins are particularly important for the functionality of the photosynthetic apparatus. As a result of induced changes in structure and functions of some intrinsic protein complexes of membranes, cell functions are disturbed. Long-term soil water deficit noticeably changes the content of chlorophylls (Chl) *a* and *b* and carotenoids and chlorophyll *a/b* ratio in leaves of plants with different tolerance to drought [10]. The functions of membrane proteins are influenced by the lipid matrix in which they are embedded, and alterations in bulk membrane lipids perturb all functions by inducing changes in the structure and function of thylakoid membrane protein complexes [9, 11].

The aim of this study was to investigate changes in structural and functional characteristics of chloroplast thylakoids in drought-resistant and drought-sensitive wheat varieties under water deficit. For these purposes two bread wheat genotypes with contrasting architectonics and different genetically stipulated sensitivities to drought were used. This approach allows us to identify not only precise bounds of variation of plant reaction to stress, but also to reveal specific features typical for highly resistant genotypes that may be taken into consideration in crop breeding practice for development of drought-tolerant varieties.

MATERIALS AND METHODS

We used two bread wheat cultivars (*Triticum aestivum* L.) contrasting in architectonics and differing in drought resistance, i.e. cv. Giymatli-2/17 (short stature, broad and drooping leaves, grain yield of 7-8 tons/ha, drought sensitive) and cv. Azamatli-95 (short stature, vertically oriented small leaves, grain yield of 8-9 tons/ha, drought tolerant) in the experiments. The different sensitivities of these cultivars to drought had been determined during some years in different regions of Azerbaijan based on grain yield [3, 4]. The plants were provided by the Experimental Station of the Agricultural Research Institute. Both genotypes were grown under field conditions in a wide area under normal water supply and under dry land conditions. Dehydration was imposed by withholding water supply. Roots and shoots were separated, fresh weight was recorded, and samples were taken for dry weight measurements. Three different samples for each treatment were taken and analyzed twice.

Leaf relative water content was estimated gravimetrically according to the method of Tambussi et al. [12].

Leaves were homogenized with a Waring blender (UK) at full speed four times for 20 sec each in an ice-cold grinding chloroplast isolation medium (1 : 6 w/v) containing 0.4 M sucrose, 20 mM Tris-HCl, 10 mM NaCl, 1 mM EDTA (sodium salt), 5 mM sodium ascorbate, and 0.1% polyethylene glycol, pH 7.8, following the procedure as described in [13]. The homogenate was filtered through four layers of cheesecloth twice. The filtrate was centrifuged at 200*g* for 5 min, and then the supernatant centrifuged at 1000*g* for 10 min. The chloroplast pellet was suspended for 30 min in a hypotonic buffer consisting of 5 mM Tris-HCl (pH 7.8) and 1 mM $MgCl_2$ and centrifuged at 5000*g* for 20 min. The pelleted thylakoid membranes were resuspended with 5 mM Tris-HCl (pH 8.0). All steps were executed at 4°C. Chlorophyll concentration was determined spectrophotometrically in 80% acetone extract [14].

For polypeptide analysis, the samples of thylakoid membranes were separated under denaturing conditions at 2-3°C in the presence of 0.1% (w/v) SDS using a 10-25% (w/v) linear gradient of polyacrylamide gel (acrylamide/methylene-bis-acrylamide ratio, 30 : 0.8) in combination with the Laemmli buffer system [15] as described previously [13].

Fluorescence (*F*) at 77K was measured using a Hitachi-850 (Japan) fluorescence spectrophotometer as reported previously [16]. Chlorophyll fluorescence was excited by dark blue light with wavelength of 440 nm. Fluorescence emission spectra were corrected for the spectral sensitivity of the spectrophotometer using rhodamine B. The samples were quickly frozen in liquid nitrogen.

Photochemical activities of chloroplasts isolated from control and drought-stressed plants were followed polarographically as O_2 evolution or uptake using a water-jacketed Clark type oxygen electrode chamber [17]. Chlorophyll concentrations equivalent to 100 μg were used for all measurements. PS II activity ($H_2O \rightarrow K_3Fe(CN)_6$) was measured in medium containing 330 mM sorbitol, 40 mM Hepes-NaOH, pH 7.6, 10 mM NaCl, and 5 mM $MgCl_2$ using 0.5 mM $K_3Fe(CN)_6$ as terminal electron acceptor. PS I activity ($DCPIP{\cdot}H_2 \rightarrow MV$) was assayed in reaction mixture (2 ml) containing 80 mM sucrose, 30 mM Tris-HCl, pH 8.0, 10 mM NaCl, 10 mM $MgCl_2$, 1 mM sodium ascorbate, and 2 μM 3-(3-4-dichlorophenyl)-1,1-dimethylurea (to block electron transport from PS II), using 0.3 mM 2,6-dichlorophenol-indophenol (DCPIP) as electron donor and 50 μM methyl viologen (MV) as electron acceptor. Photochemical activities were assayed in μmol O_2/mg Chl per hour.

Photoinduced changes of fluorescence yield were measured at room temperature using laboratory-built instrument as described earlier [18]. Potential quantum yield of PS II was estimated according to the formula:

$$\Phi_p = F_v/F_m = (F_m - F_0)/F_m.$$

RESULTS AND DISCUSSION

Significant differences in relative water content were observed between normally irrigated plants and in those

subjected to drought stress. The Giymatli-2/17 genotype grown with normal water supply conditions showed higher relative water content (RWC) in the leaves. Drought-stress conditions induced a slightly larger decrease in RWC in the more sensitive cv. Giymatli-2/17 than in the more tolerant cv. Azamatli-95; dehydration decreased the RWC by 14% in comparison with fully irrigated plants. The rate of water loss during drought was low in Azamatli-95. The RWC decreased from 83.9 to 72.1% following stress. Exposure to drought caused a reduction in dry weight accumulation in Giymatli-2/17 plants relative to control, whereas it had smaller, insignificant effects in cv. Azamatli-95.

A reduction in the total Chl content and Chl *a*/*b* ratio occurred during drought stress (Fig. 1). This pattern of change was not evident in the tolerant genotype Azamatli-95, in which changes in these parameters were not statistically significant, whereas the difference was significant in sensitive cv. Giymatli-2/17 in relation to control. A drought-induced decrease in pigment contents was previously reported in several plant species including pea [19], durum wheat [6], and *Boea hydroscopica* [9]. The more drought-sensitive cv. Giymatli-2/17 showed a slight increase in the pool size of xanthophyll cycle components, but this effect was not shown in the tolerant cv. Azamatli-95.

Figure 2 shows density patterns of membrane proteins of two wheat genotypes with different tolerance to drought and contrasting architectonics. As shown in the figure, thylakoid membranes isolated from the wheat genotypes grown under normal water supply appeared to have about 26 polypeptides with molecular weights from 115 to 11 kDa. It was found that Giymatli-2/17 genotype with broad and drooping leaves and drought-sensitive is

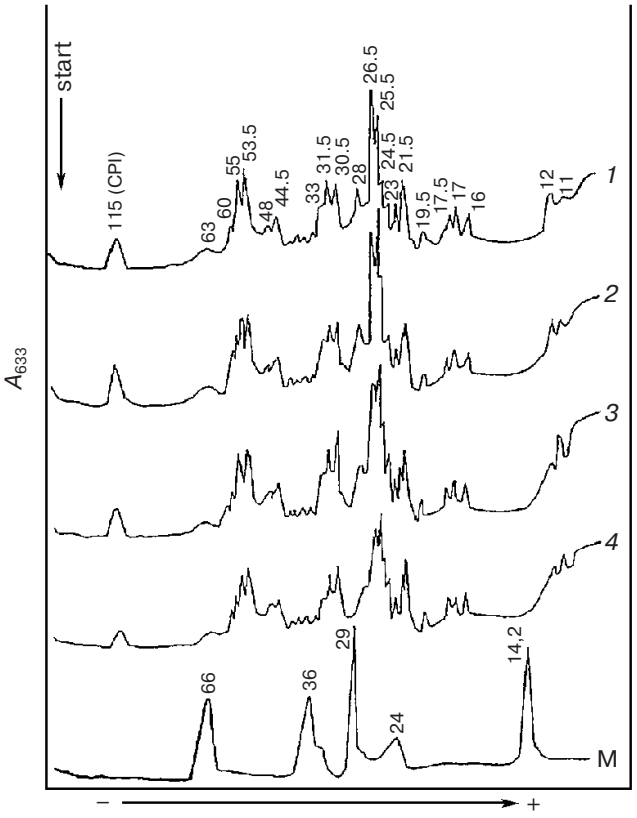

Fig. 2. Density patterns from Coomassie blue staining SDS-PAGE (10-25% gel) analysis of thylakoid membrane proteins from wheat plants grown in field conditions under normal water supply (Azamatli-95 (*1*) and Giymatli-2/17 (*3*)) and drought stress (Azamatli-95 (*2*) and Giymatli-2/17 (*4*)). M, standard proteins (kDa): bovine serum albumin (66), glyceraldehydes-3-phosphate dehydrogenase (36), carbonic anhydrase (29), trypsinogen (24), trypsin inhibitor (20.1), and α-lactalbumin (14.2). Samples applied to each well correspond to 50 μg protein.

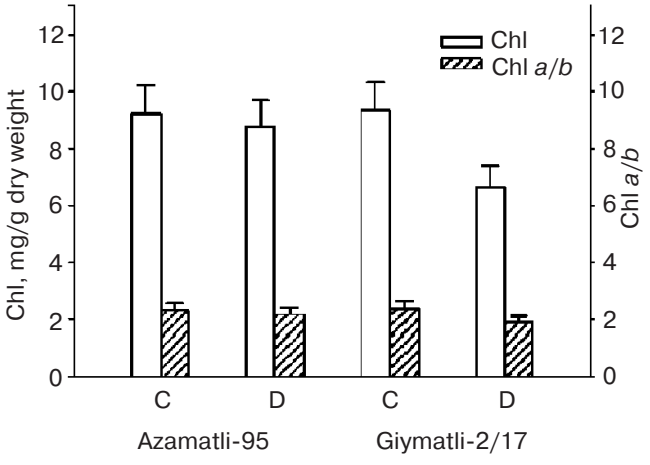

Fig. 1. Chlorophyll content of leaves of wheat genotypes Azamatli-95 and Giymatli-2/17 during water deficit. C, control; D, drought stress. Results are means of three repetitions of two independent experiments (mean ± SE, $n = 6$).

characterized by low content of chlorophyll *a*-protein of PS I core (CP I) and β-subunit of CF_1 ATP-synthase complex, high content of proteins in the 33-30.5 kDa region, and relatively high amount of polypeptides of light-harvesting complex under normal irrigation in comparison with drought-tolerant genotype Azamatli-2/17, having vertically oriented small leaves. Drought stress caused significant changes in the content and composition of thylakoid membrane proteins. The content of CP I (115 kDa) and apoprotein P700 (63 kDa) was maintained at relatively high level in tolerant cv. Azamatli-95 but was slightly affected by drought in more sensitive cv. Giymatli-2/17. It is interesting to note that the content of 60 kDa polypeptide strongly increases (about 2-fold) in the drought-resistant cv. Azamatli-95. However, detection of this polypeptide was not possible in experiments with seedlings of wheat grown in a growth chamber under controlled environment conditions. On the basis of these results and literature data, we suggest that this protein is related to dehydrins (PCA 60). Seasonal expression of

dehydrins has been noted in several species [20]. The dehydrin family of proteins is induced by environmental stresses that result in cellular dehydration [21]. All these protein groups are characterized with high hydrophilicity. During dehydration of cells, they prevent water loss on account of high hydrophilic capacity and stabilize cell proteins. PCA 60 was distributed in the cytosol, plastid, and nucleus. Although the functional role of dehydrins remains speculative, the data support their role in preventing denaturation of proteins exposed to dehydration stress in a manner similar to chaperones.

The synthesis of α- and β-subunit of CF_1 ATP synthase complex (55 and 53.5 kDa, respectively) tended to increase slightly in stressed plants of Azamatli-95 and to decrease in cv. Giymatli-2/17. The low content of β-subunits of CF_1 ATP-synthase complex has been also shown in pea plants subjected to water deficit at high light exposure [22, 23]. Steady-state levels of the core antenna of PS II (CP 47 and CP 43) serving as the connecting antenna between the main light harvesting complex LHC II and the reaction center of PS II remained more or less unchanged in the investigated plants. These results agree with data that were obtained early [22-24].

The most striking change was the appearance of a protein with molecular mass of 40.5 kDa in plant leaves. It is absent in leaves from non-stressed plants, but at a low level it was detected in only the tolerant plants subjected to water deficit. According to the current literature, C40.4 protein shares high sequence homology with CDSP (chloroplast drought-induced stress protein), the previously described accumulation of a 34 kDa thylakoid protein in tomato in response to drought. Substantial increases in CDSP 34 transcript content and protein abundance were also observed in potato plants subjected to high illumination [25]. The accumulation of two chloroplast nuclear-encoded proteins in water-stressed *Solanum tuberosum* plants was reported [26]. A stromal protein of 32 kDa related to thioredoxins was suggested to maintain the redox state of chloroplast proteins upon drought stress [27]. The other protein of 34 kDa, named CDSP 34, is proposed to participate in structural stabilization of thylakoids upon environmental constraints and prevent damage resulting from osmotic or oxidative stress. It is supposed that C40.4 protein is closely bound with LHC II and has a functional role by modulating photosynthetic effectiveness and light dissipation of excess absorbed light energy inside antenna complex [28].

At the same time, in the more sensitive cv. Giymatli-2/17 there was a considerable decrease in amounts of proteins with molecular weight 33-30.5 kDa. The decrease in the content of 32-kDa protein (D1-protein of reaction center of PS II) in thylakoid membrane from water-stressed plants (especially in cv. Giymatli-2/17) seems to be due in part to its enhanced degradation rate [29]. High rate of D1-protein turnover provides stability of thylakoid membranes and their electron transport chain to damaging action of free radicals forms under stress conditions. On the other hand, thylakoid membranes from stressed plants showed an increased level of LHC polypeptides (28-24.5 kDa) in tolerant cv. Azamatli-95 compared to Giymatli-2/17, at which the level of these units decreases.

A slight increase in 21.5-kDa polypeptide (according to literature data, it related to WSCP (water-soluble chlorophyll proteins)) was also observed in both genotypes under drought. It is supposed that this protein might be involved in a decrease in protease activity in leaf senescence. Correlation between tolerance and overexpression of some proteins including those of 60, 40.5, and 28-24.5 kDa assumes that changes in expression of their genes can be functionally involved in the ability of plants to survive and grow under water deficiency.

Table 1 presents data of membrane proteins in which changes in content are significant under drought stress.

In parallel, we also measured the fluorescence emission spectra (77K) of chloroplasts from normally irrigated and drought-stressed plants. As shown in Fig. 3, chloroplasts from drought-sensitive cv. Giymatli-2/17 have more intensive fluorescence at 740 nm from PS I under normal water supply. The F687/F740 ratio of con-

Table 1. Photosynthetic membrane proteins from wheat chloroplasts subjected to changes under drought stress

Samples	Molecular mass of proteins, kDa*					
Azamatli-95 (control)	CP I, 115 (PS I core)	60	55 and 53.5 (α- and β-subunits of CF_1)	33-30.5	28-24.5 (proteins of LHC)	21.5
Azamatli-95 (drought)	0	+	+	0	+	+
Giymatli-2/17 (control)	CP I, 115 (PS I core)	60	55 and 53.5 (α- and β-subunits of CF_1)	33-30.5	28-24.5 (proteins of LHC)	21.5
Giymatli-2/17 (drought)	–	0	–	–	–	+

* (+), protein content increases; (–), content decreases; 0, no changes.

trol (non-drought stressed) chloroplasts of cv. Azamatli-95 was close to 0.38 and for Giymatli-2/17 – 0.35. A shift of the main peak from 742 to 740 nm (in Azamatli-95) and from 740 to 738 nm (in Giymatli-2/17) is observed in both plants grown under water deficit. The short wavelength peaks at 687 and 695 nm (fluorescence from the PS II core complex CP 47 and CP 43) remained, and their fluorescence intensities started to increase sharply under water deficit. This is especially observed in drought-sensitive cv. Giymatli-2/17. At the same time, in chloroplasts from stressed plants the F687/F740 ratio rises compared with control plants; the lowest value was that of the Azamatli-95 (F687/F740 = 0.45) and the highest of Giymatli-2/17 (F687/F740 = 0.77), suggesting again that the most detrimental influence of drought stress occurs in Giymatli-2/17.

The results suggest that the antenna system of the photosynthetic apparatus in the drought-tolerant cv. Azamatli-95 is rapidly reorganized, and plants began to adapt to environmental stress. It is possible that antenna pigments of the plants move much more from the reaction centers into the lipid bilayer of thylakoid membranes for display of the protective mechanism of plants on survival under drought conditions. More frequently changes in F687/740 ratio can be explain by redistribution of excitation light energy between PS II and PS I.

Significant differences were found in the functional activity of the photosynthetic apparatus at the level of photochemical reactions of chloroplasts in comparative studies of genotypes distinguished by architectonics and drought resistance. In our experiments, the highest PS II activity (oxygen evolution rate) of irrigated plants was found in the drought-sensitive cv. Giymatli-2/17 with broad and drooping leaves (Table 2). The electron transport activities of both stressed plants were lower than in the control plants. However, the activity of PS II was significantly affected by dehydration in Giymatli-2/17 plants – only 41% of control value remained. In drought-stressed cv. Azamatli-95 leaves, the photochemical activity of PS II was about 78% of the control value. The cause of PS II inactivation in both genotypes might be suppression of synthesis of 32 kDa protein, which is a carrier of photochemically active forms of Chl *a*, or breach of electron transfer from pheophytin – an intermediate electron carrier on quinone acceptor (Q_A) in non-cyclic transport of electrons. It is possible that desiccation inhibited the energy transfer from the Chl molecules anchor to PS II core complexes. PS I activity (O_2 uptake rate), however, was affected much less under drought stress (Table 2). This can be due to a higher ability of PS I to adapt to dehydration.

Values of fluorescent parameters that characterize the functional state of the photosynthetic apparatus of winter wheat plants grown under different conditions of water regime are shown in Table 3. The potential quantum yield of photochemical reactions of PS II (F_v/F_m ratio) in chloroplasts from control (non-drought stressed) plants was 0.74 for Azamatli-95 and 0.81 for Giymatli-2/17, that is typical for normally grown plants. As it seems from Table 3, the state of PS II in dehydration stress was significantly changed. Potential yield of photochemical reactions of PS II undergoes appreciable changes in comparison with control plants; the highest value of F_v/F_m was in Azamatli-95 (F_v/F_m = 0.71) and the lowest in Giymatli-2/17 (F_v/F_m = 0.69). It is interesting to note that chloroplasts from non-drought-tolerant genotype Giymatli-2/17 have higher photochemical efficiency of

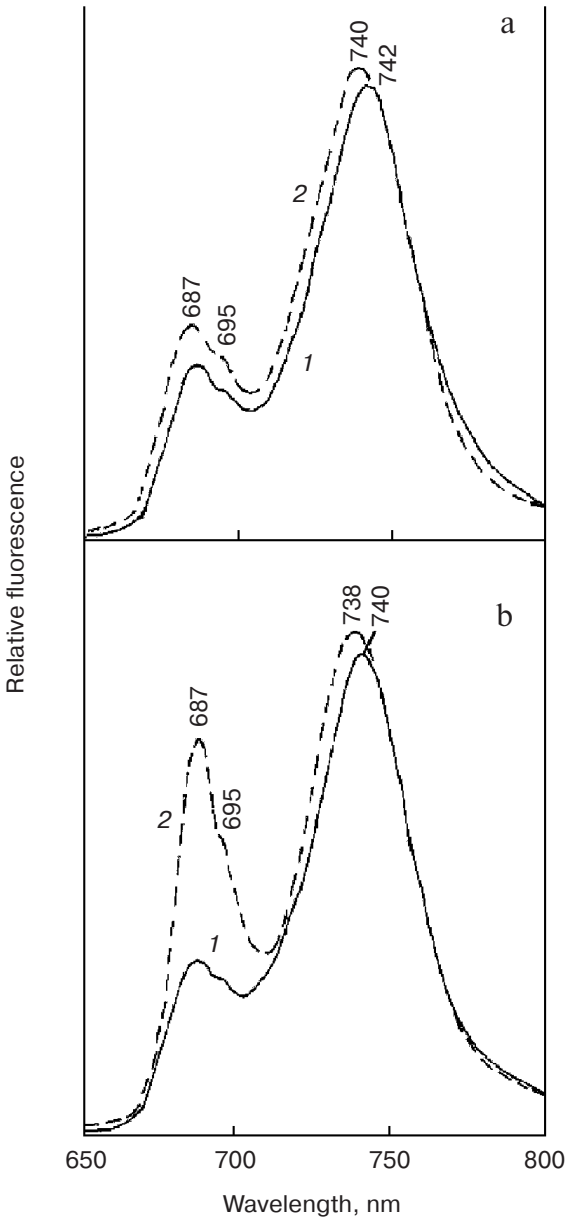

Fig. 3. Fluorescence emission spectra at 77K of chloroplasts from drought-tolerant Azamatli-95 (a) and drought-sensitive Giymatli-2/17 (b) plants grown under normal water supply (*1*) or drought conditions (*2*).

Table 2. PS II and PS I activity in chloroplasts from wheat genotypes subjected to drought stress (μmol O_2/mg Chl per hour)

Cultivar	PS II $H_2O \rightarrow K_3Fe(CN)_6$	in %	PS I DCPIP·H → MV	in %
Azamatli-95 (control)	45 ± 4	100	250 ± 12	100
Azamatli-95 (drought)	35 ± 3	78	225 ± 9	90
Giymatli-2/17 (control)	85 ± 7	100	190 ± 8	100
Giymatli-2/17 (drought)	35 ± 4	41	150 ± 4	79

Table 3. Change in parameters of chlorophyll *a* fluorescence in chloroplasts isolated from wheat leaves after drought. Fluorescence components: F_0, constant fluorescence; F_v, variable fluorescence; F_m, maximal fluorescence*

Variant	Control	Drought	% of control
Azamatli-95			
F_0	29.0 ± 1.2	30.0 ± 2.8	103
F_v	85.0 ± 6.1	74.5 ± 5.4	87
F_v/F_m	0.74	0.71	96
Giymatli-2/17			
F_0	27.0 ± 1.1	28.5 ± 2.9	106
F_v	118.0 ± 6.5	63.0 ± 4.3	54
F_v/F_m	0.81	0.69	85

* Average arithmetic and standard errors from three independent experiments, each of which was carried out in double biological replicates, are shown in the table.

PS II under regular irrigation conditions of growth. However, low ratio of F_v/F_m again confirms that strong effect of drought appeared in cv. Giymatli-2/17 (cv. Giymatli-2/17 is strongly affected by drought). Decreasing of photochemical efficiency (F_v/F_m) under severe drought can be considered as a fact of damage of photosynthetic reaction centers.

Both Q_B-reducing and Q_B-non-reducing complexes of PS II make a contribution to variable fluorescence (F_v). Charge separation is realized in Q_B-non-reducing complexes of PS II, but electrons are not transported to the plastoquinone pool. Q_B-reducing complexes of PS II in active state are able to transport electrons between Q_A and Q_B. They lose this ability when D1-protein is damaged and become Q_B-non-reducing complexes [30]. Under optimal conditions due to reactions of a reparation cycle, a constant ratio between these types of complexes of PS II is supported. Dehydration probably induces disruption of reactions at the acceptor side of PS II, expressed in increase in number of Q_B-non-reducing centers.

So, drought induced photoinhibition and photodestruction of pigments and pigment–protein complexes and destabilization of photosynthetic membrane. In cv. Azamatli-95, the lack of changes in pigment content and composition following drought indicated the capacity to preserve the photosynthetic apparatus. The higher water content and its better distribution in the stressed cv. Azamatli-95 permitted the plants to retain a higher turgor in comparison with cv. Giymatli-2/17, which resulted in maintained growth, induced photoinhibition, and photodestruction of pigments and pigment–protein complexes and destabilization of photosynthetic membrane. In cv. Azamatli-95, the lack of changes in pigment content and composition following drought indicated the capacity to preserve the photosynthetic apparatus. At the same time, the decline in PS II activity induced by water deficit was more marked in sensitive cv. Giymatli-2/17 than in tolerant cv. Azamatli-95. The more drought-sensitive cv. Giymatli-2/17 responded to a period of stress by reducing photosynthetic efficiency and biomass accumulation. Therefore, in cv. Azamatli-95 the photosynthetic electron transport was probably sufficient to preclude the buildup of excess energy in PS II [6]. On the other hand, drought tolerant cv. Azamatli-95 seems able to avoid drought stress by maintaining a high photosynthetic activity and does not suffer from oxidative stress high enough to trigger the defense mechanisms active in cv. Giymatli-2/17.

REFERENCES

1. Bray, E. A. (1997) *Trends Plant Sci.*, **2**, 48-54.
2. Rizhsky, L., Liang, H., and Mittler, R. (2002) *Plant Physiol.*, **130**, 1143-1151.
3. Aliev, J. A. (1998) *Photosynthesis: Mechanisms and Effects* (Garab, G., ed.) Vol. 5, Kluwer Academic Publishers, Dordrecht-Boston-London, pp. 3829-3832.
4. Aliev, J. A. (2001) *Wheat in a Global Environment* (Bedo, Z., and Lang, L., eds.) Vol. 9, Kluwer Academic Publishers, Dordrecht-Boston-London, pp. 693-698.
5. Sherwin, H. W., and Farrant, J. M. (1998) *Growth Regul.*, **24**, 203-210.
6. Loggini, B., Scartazza, A., Brugnoli, E., and Navari-Izzo, F. (1999) *Plant Physiol.*, **119**, 1091-1098.
7. Asada, K. (2006) *Plant Physiol.*, **141**, 391-396.
8. Breusegem, F., and Dat, J. (2006) *Plant Physiol.*, **141**, 384-390.
9. Navari-Izzo, F., Quartacci, M. F., Pinzino, C., Rascio, N., Vazzana, C., and Sgherri, C. L. M. (2000) *Plant Physiol.*, **124**, 1427-1436.
10. Mi, H.-L., Xu, X., Li, S.-H., He, J., Zhang, Y.-P., Zhao, T.-C., and Ma, Y.-M. (2004) *Acta Bot. Boreali-Occident. Sci.*, **24**, 1816-1821.
11. Quartacci, M. F., Pinzino, C., Sgherri, C. L. M., and Navari-Izzo, F. (1995) *Plant Physiol.*, **108**, 191-197.
12. Tambussi, E. A., Nogues, S., and Araus, J. L. (2005) *Planta*, **221**, 446-458.
13. Aliyev, J. A., Suleymanov, S. Y., Guseinova, I. M., Asadov, A. A., and Ismailov, M. A. (1992) *Biokhimiya*, **57**, 679-686.
14. Mc-Kinney, G. (1941) *J. Biol. Chem.*, **140**, 315-322.
15. Laemmli, U. K. (1970) *Nature*, **227**, 680-685.
16. Asadov, A. A., Zulfugarov, I. S., Suleymanov, S. Y., and Aliyev, J. A. (1986) *DAN SSSR*, **287**, 444-447.
17. Guseinova, I. M., Suleymanov, S. Y., and Aliyev, J. A. (2001) *J. Biochem. Mol. Biol.*, **34**, 496-501.
18. Klimov, V. V., Allakhverdiev, S. I., Shuvalov, V. A., and Krasnovsky, A. A. (1982) *FEBS Lett.*, **148**, 307-312.
19. Moran, J. F., Becana, M., Iturbe-Ormaetyl, I., Frechilla, S., Klucas, R. V., and Aparicio-Tejo, P. (1994) *Planta*, **194**, 346-352.
20. Wisnewski, M., Webb, R., Balsamo, R., Close, T. J., Yu, X-M., and Griffith, M. (1999) *Physiol. Plant.*, **105**, 600-608.
21. Close, T. J. (1997) *Physiol. Plant.*, **100**, 291-296.
22. Giardi, M. T., Cona, A., Kucera, T., Masojidek, J., and Mattoo, A. K. (1995) in *Proc. Int. Congr. Integrated Studies on Drought Tolerance of Higher Plants "Inter Drought 95"*, pp. 1-5.
23. Guseynova, I. M., Suleymanov, S. Y., and Aliyev, J. A. (2006) *Biochemistry (Moscow)*, **71**, 173-177.
24. Masojidek, J., Trivedi, S., Halshaw, L., Alexiou, A., and Hall, D. O. (1991) *Plant Physiol.*, **96**, 198-207.
25. Gillet, B., Beyly, A., Peltier, G., and Rey, P. (1998) *The Plant J.*, **16**, 257-262.
26. Pruvot, G., Cuine, S., Peltier, G., and Rey, P. (1996) *Planta*, **198**, 471-479.
27. Rey, P., Pruvot, G., Becuwe, N., Eymery, F., Rumeau, D., and Peltier, G. (1998) *Plant J.*, **13**, 97-107.
28. Monte, E., Ludevid, D., and Prat, S. (1999) *The Plant J.*, **19**, 399-410.
29. Sippola, K., Kanervo, E., Murata, N., and Aro, E.-M. (1998) *Eur. J. Biochem.*, **251**, 641-648.
30. Pshibytko, N. L., Kalitukho, L. N., and Kabashnikova, L. F. (2003) *Russ. J. Plant Physiol.*, **50**, 51-58.