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Biochimica et Biophysica Acta 1767 (2007) 869–875
Biochimica et Biophysica Acta
BBA
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Structural–functional state of thylakoid membranes of wheat genotypes under water stress

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Received 9 October 2006; received in revised form 21 December 2006; accepted 22 January 2007
Available online 28 January 2007

Abstract

Plants were grown in field conditions in the wide area under normal water supply and severe water deficit. Two wheat (*Triticum aestivum* L.) genotypes contrasting by architectonics and differing in drought-resistance were used: Giymatli-2/17, short stature, with broad and drooping leaves, drought-sensitive, and Azamatli-95, short stature, with vertically oriented small leaves, drought-tolerant). It was found out that Giymatli-2/17 was characterized by relatively low content of Chl a-protein of PS I (CP I) and β-subunit of ATP-synthase complex, the high content of proteins in the 33–30.5 kDa region and LHC polypeptides (28–24.5 kDa), the intensive fluorescence at 740 nm and more high photochemical activity of PS II under normal irrigation compared with Azamatli-95. However, the content of CP I (M_r 115 kDa) and apoprotein of P700 with M_r 63 kDa insignificantly increases in the drought-resistant genotype Azamatli-95 under extreme water supply condition while their content decreases in drought-sensitive cv Giymatli-2/17. Intensity of synthesis α- and β-subunits of CF_1 (55 and 53.5 kDa) also decreases in Giymatli-2/17. The levels of the core antenna polypeptides of FS II with M_r 46 and 44.5 kDa (CP47 and CP43) remains stable both in normal, and stressful conditions. At the same time the significant reduction is observed in the content of polypeptides in the 33–30.5 kDa region in the more sensitive genotype Giymatli-2/17. There is an increase in the LHC II polypeptides level in tolerant genotype Azamatli-95 in contrast to Giymatli-2/17 (where the content of these subunits is observed decreasing). The intensity of short wavelength peaks at 687 and 695 nm sharply increases in the fluorescence spectra (77 K) of chloroplasts from sensitive genotype 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.

Keywords: Drought; Wheat genotype; Thylakoid membrane; Polypeptide; Fluorescence; Adaptation

1. Introduction

Under natural conditions, plants are frequently subjected to external stresses which 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 plant to adapt to stress and maintain its 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 abotic stress limiting its grain yield [3,4].

The most important phenomena in drought stress is the deleterious effect that can be seen in the photosynthetic machinery of the plant cells.

Water stress, like most environment constraint, generates reactive oxygen species (ROS) within chloroplast due to excess excitation energy in relation to limited CO_2 fixation capacity [5–7]. Reactive oxygen causes damage such as protein modi-

Abbreviations: ROS, reactive oxygen species; RWC, relative water content; PS I, photosystem I; PS II, photosystem II; LHC, light-harvesting chlorophyll a/b-protein complex; Chl, chlorophyll; SDS, sodium dodecyl sulphate; F, fluorescence; DCIP, 2,6-dichlorophenolindophenol; MV, methyl viologen; DCMU, 3(3-4-di-chlorophenyl)-1,1-dimethylurea

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doi:10.1016/j.bbabio.2007.01.014

fication [8], lipid peroxidation [9]. To prevent reactive oxygen formation, plants evolved mechanisms, such as heat dissipation of excess energy through carotenoids. Furthermore, chloroplastic antioxidants and enzymes allow scavenging of reactive oxygen species [5,10]. Reactivated oxygen also reacts with proteins, which become more prone to proteolysis [11] and there is some evidence that Chl breakdown may be a prerequisite to the degradation of associated proteins [12].

Photosynthetic electron transfer reactions that take place in thylakoid membranes have been shown to be a remarkably resistant to dehydration [13,14], suggesting that higher plants developed adaptive mechanisms conferring dehydration tolerance to the photosynthetic apparatus. In response to drought, the adaptation shown by many plants could partly be due to changes in membrane composition and phase behavior, which optimizes the fluidity [15]. Indeed, models for thylakoid membrane function require mobility of protein components and redox carriers. Membrane proteins are particularly important for the functionality of the photosynthetic apparatus. The functions of membrane proteins are influenced by the lipids matrix in which they are embedded. Within the thylakoids the membrane lipids have an important role to play in stabilizing the structural arrangement and, via the lipid–protein complexes and possibly in maintaining their spatial distribution [16]. Alterations in bulk membrane lipids perturb all function by inducing changes in the structure and function of thylakoid membrane protein complexes [17,18].

Changes in gene expression are fundamental to the responses that occur during water deficit and numerous drought-induced genes have been characterized [19]. Therefore, linking the expression of a gene to a high degree of tolerance within a genotype might provide arguments for its role in adaptation [20]. Until now, very few drought-induced chloroplastic proteins have been identified in detail, and the molecular basis of drought-stress tolerance is still unknown.

The aim of this study was to investigate structural and functional characteristics of thylakoid membrane that ensures resistance of plant organisms to water deficit. For these purposes two bread wheat genotypes with contrast architectonics and different genetically stipulated sensitivities to drought were used. Such approach allows to identify not only precise bounds of variation of plant reaction to stress, but also to reveal specific features typical for high-resistant genotypes that may be taken into consideration in crop breeding practice for development of drought tolerant varieties.

2. Materials and methods

2.1. Plant material

In the experiments we used two bread wheat genotypes *(Triticum aestivum* L.), contrasting by architectonics and differing in drought resistance, cv Giymatli-2/17—short stature, with broad and drooping leaves and grain yield of 7–8 $t \times ha^{-1}$, drought sensitive, cv Azamatli—short stature, with vertically oriented small leaves and grain yield of 8–9 $t \times ha^{-1}$, drought tolerant. All genotypes were grown in field conditions in the wide area under normal water supply and dryland conditions from November to May. The plants were provided by Experimental Station of the Research Institute of Agriculture (Baku, Azerbaijan). Different sensitivities of these genotypes to drought had been determined during some years in different regions of Azerbaijan based on grain yield [3,4]. A group of plants from both cultivars was cultivated under optimum irrigation condition (control), and another set of plants was subjected to water deficit. Dehydration was imposed by withholding water supply. Samples were collected from control and stressed plants at grain filling period up 9^{30} to 10^{30}. 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.

2.2. Relative water content

Leaf relative water content (RWC) was estimated gravimetrically according to the method of Tambussi et al. [21].

2.3. Isolation of thylakoid membranes

Leaves were homogenized with a Waring blender at full speed four times for 20 s each in an ice-cold grinding chloroplast isolation medium (1: 6 w/v) containing 0.4 M sucrose, 20 mM Tris, 10 mM NaCl, 1 mM EDTA (sodium salt), 5 mM sodium ascorbate, and 0.1% polyethylene glycol, pH 7.8 following the procedure of Aliev et al. [22]. 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 8.0) 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.

2.4. Chlorophyll and protein determinations

The chlorophyll concentration was determined in 80% acetone extract [23]. Protein determination was performed according to [24]. Samples frozen in liquid nitrogen and stored at −80 °C until required.

2.5. Electrophoresis analysis

For polypeptide analysis, the samples of thylakoid membranes were separated under denaturing conditions at 2 to 3° in the presence of 0.1% (w/v) SDS using a 10 to 25% (w/v) linear gradient polyacrylamide gel (acrylamide: methylenebisacrylamide ratio=30:0.8) in combination with the Laemmli buffer system [25] as described previously [26]. To each slot 20 to 45 μl of samples (an equal Chl content) were applied. The gels were stained for 30 min with 0.04% (w/v) Coomassie brilliant blue G-250 (France) prepared in 3.5% perchloric acid ($HClO_4$). Immediately after electrophoresis the gels were scanned using an Ultroscan 2202 densitometer (LKB, Sweden) with a 633 nm laser as the light source. A set of standard proteins (kDa) consisting of bovine serum albumin (66), glyceraldehyde-3-phosphate dehydrogenase (36), carbonic anhydrase (29), trypsinogen (24), trypsin inhibitor (20.1), and lactalbumin (14.2) (Sigma, USA) was used for the determination of the molecular masses of polypeptides.

2.6. Chl fluorescence of chloroplasts

The measurements of fluorescence (F) at 77 K were performed using a Hitachi-850 (Japan) fluorescence spectrophotometer as reported previously [27]. Fluorescence emission spectra were corrected for the spectral sensitivity of the spectrophotometer using rhodamine B. The samples on quartz glass fiber were quickly frozen at 77 K by dipping the glass fiber into liquid nitrogen.

2.7. Assay of electron transport activities

Electron transport activities of chloroplasts isolated from control and drought-stressed plants were followed polarographically as O_2 evolution or uptake at 20 °C using a water-jacketed Clark type oxygen electrode chamber under illumination with saturating white actinic light (850 $\mu E\ m^{-2}\ s^{-1}$), according to [22]. Chloroplast concentrations equivalent to 100 μg Chl were used for all measurements. Artificial donors and acceptors were added imme-

diately before or during illumination. The following electron transport activities were assayed in μmol $O_2 \cdot mg^{-1}$ Chl·h^{-1}. PS II activity ($H_2O \rightarrow K_3Fe(CN)_6$) was measured in a 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 was assayed in the reaction mixture contained in 2 ml, 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-di-chlorophenyl)-1,1-dimethylurea (in order to block electron transport from PS II), using 0.3 mM 2,6-dichlorophenolindophenol as electron donor and 50 μM methylviolgen as electron acceptor.

3. Results and discussion

The investigated genotypes respond to water deficit through various changes in physiological and biochemical processes.

Significant differences in relative water content (RWC) were observed between normally irrigated plants and in those subjected to water stress (Fig. 1). The Giymatli-2/17 genotype grown in normal water supply condition showed the higher 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 lowered from 83.9% to 72.1% following stress. Exposure to drought caused a reduction in dry weight accumulation in Giymatli-2/17 plants, whereas it had smaller, insignificant effects in cv. Azamatli-95, even though both cultivars showed a certain drop in RWC.

A reduction in the total chlorophyll content and Chl a/b ratio occurred during drought stress (Fig. 2). This pattern of change was not evident in tolerant genotype Azamatli-95, in which these parameters did not change statistically, whereas the difference was significant in sensitive cv. Giymatli-2/17. A drought-induced decrease in pigment contents was previously reported in several plant species, including pea [28], durum wheat [7] and *Boea hydroscopica* [29]. The more drought-sensitive cv. Giymatli-2/17 showed a slight increase in the pool size of xantophyll-cycle components, but such effect was not shown in the tolerant cv. Azamatli-95, which may be explained by its higher rate of electron transport compared with cv. Giymatli-2/17 [30].

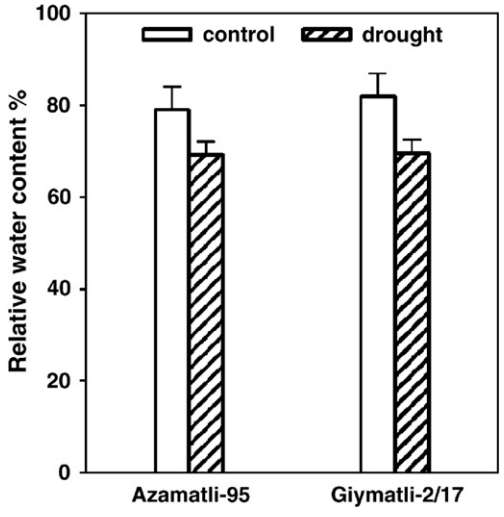

Fig. 1. Effect of water stress on relative water content of leaves in normally irrigated and drought stressed plants of *Triticum aestivum* wheat. Vertical bars represent SE of mean, $n=6$.

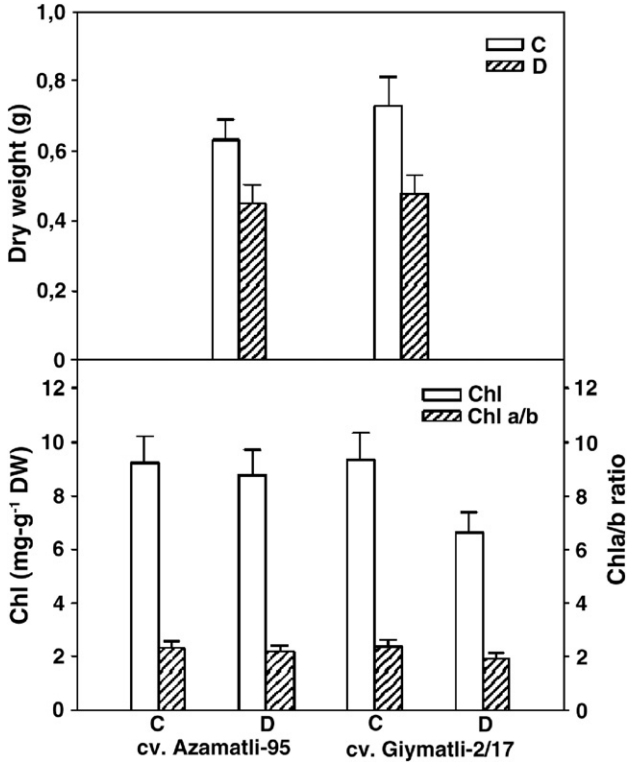

Fig. 2. Dry weight and chlorophyll content of leaves of the wheat genotypes Azamatli-95 and Giymatli-2/17 during water deficit; C—control; D—drought stress. Results are the means of three repetitions of two independent experiments SE±($n=6$).

Total protein synthesis was slightly reduced by water deficit in these experiments. The decrease of thylakoid proteins observed during dehydration may be associated with degradation of lipoprotein thylakoid membrane structure [29]. In addition, the photosynthetic apparatus may show acclimation responses such as changes in the relative proportion of stacked and unstacked membrane domains [31]. At the ultrastructural level the thylakoid system of hydrated chloroplasts was organized in several well-defined and regularly distributed grana connected by parallel stroma lamellae. The increased thylakoid stacking in dried chloroplasts could be a consequence of membrane and/or environmental changes leading to a weakening of the repulsive force between the membrane surfaces [32]. Another influential factor might be the rise due to water loss, the stroma ionic charge screening the repulsive force between thylakoids [32].

The protein profiles of thylakoid membranes in nonstressed and water-stressed plants were analyzed. Fig. 3 shows density patterns from Coomassie blue staining SDS-PAGE analysis of membrane proteins of two wheat genotypes with different tolerance to drought and contrast architectonics. As shown in Fig. 3, thylakoid membranes isolated from the wheat genotypes grown under normal water supply appeared to have about 26

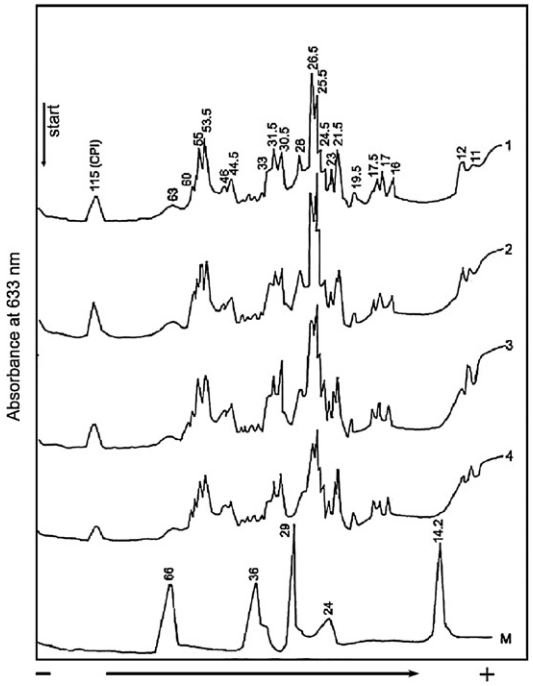

Fig. 3. Density patterns from Coomassie blue staining SDS-PAGE (10–25% gel) analysis of thylakoid membrane proteins from wheat plants grown in field conditions under normal water supply (Azamatli-95) (1) and Giymatli-2/17 (3)) and drought stress (Azamatli-95) (2) and Giymatli-2/17 (4)). M, standard proteins (kDa): bovine serum albumin (66), glyceraldehydes-3-phosthate dehydrogenase (36), carbonic anhydrase (29), trypsinogen (24), and α-lactalbumin (14.2). Samples on an equal Chl content (25 μg) were placed on the gel.

polypeptides with M_r from 115 to 11 kDa. It was found out that Giymatli-2/17 genotype with broad and lodging leaves and drought-sensitive is characterized by low content of chlorophyll a-protein of photosystem (PS) I core (CP I) and β-subunit of CF_1 ATP-synthase complex, the high content of proteins in the 33–30.5 kDa region and the relative high amount of polypeptides of light-harvesting complex (LHC) 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 membranes proteins. The content of CP I (115 kDa) and apoprotein of P700 (63 kDa) were maintained at relatively high levels in tolerant genotype Azamatli-95, but were slightly little affected by drought in more sensitive cv. Giymatli-2/17. It is interesting to note that the intensity of 60 kDa polypeptide strongly increases (about 2-fold higher) in the drought-resistant genotype Azamatly-95. However, a detection of this polypeptide was not available in the experiments with seedlings of wheat grown in growth chamber under controlled environment conditions [30]. On the basis of obtained result and literature data, it is possible to suggest that this protein is related to dehydrins (PCA 60). Seasonal expression of dehydrins has been noted in several species [33]. The dehydrin family of proteins is induced by environmental stresses that result in cellular dehydration [34]. All these protein groups are characterized with high hygrophilous protein molecules. During dehydration of cells they prevent water loss on account of high hydrophilic capacity and stabilize cell proteins. PCA 60 was freely distributed in the cytosol, plastid, and nucleus. Although the functional role of dehydrins remains speculative, the data support the hypothesis that it plays a role in preventing denaturation of proteins exposed to dehydrative stresses 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 [30,35]. 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 reaction center of PS II remained more or less unchanged in both genotypes. These results agree with data that were obtained early [30,35,36].

The most striking change was a revelation of protein with molecular mass of 40.5 kDa in tolerant genotype Azamatli-95. It is absent in leaves from nonstressed plants, but at a lower level was detected in only tolerant genotypes, subjected to water deficit. According to the current literature, C 40.4 protein share high sequence homology with GDSP (termed CDSP for chloroplastic 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 and protein abundance were also observed in potato plants, subjected to high illumination [37]. The accumulation of two chloroplastic nuclear-encoded proteins in water-stressed *Solanum tuberosum* plants was reported [38]. A stromal protein of 32 kDa related to thioredoxins was suggested to maintain the redox state of chloroplastic proteins upon drought stress [39]. Another protein of 34 kDa, named CDSP 34 protein, 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 bounded with LHC II and has functional role by modeling photosynthetic effectiveness and light dissipation of excess absorbed light energy inside antenna complex [40].

At the same time, in the more sensitive genotype Giymatli-2/17, there was a considerable decrease of amounts of proteins in 33–30.5 kDa region. The decrease in the content of 31.5 kDa protein in thylakoid membrane from water-stressed plants (especially in cv. Giymatli-2/17) seems to be due, in part, to its enhanced degradation rate [30,41]. 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 literature data it related to WSCP-water-soluble chlorophyll proteins) was also observed in both genotypes under drought. Such effect was found by us in previous researches with durum wheat seedlings under water stress [30]. It is supposed that this

protein might involve in the decrease of protease activity in leaf senescence.

Drought also caused a decrease in the synthesis of low molecular weight polypeptides of 17.5–12 kDa in both genotypes under extreme condition of water supply. The intensity of 11 kDa polypeptide slightly increased in cv. Azamatli, but significantly decreased in cv. Giymatli-2/17.

Correlation between tolerance and overexpression of some proteins including 60, 40.5, and 28–24.5 kDa assumes that changes in expression of these polypeptides genes can be functionally involved in the ability of plants to survive and grow under water deficiency.

According to the current literature, there is a cycle of PS II reparation during which the most damaged 32 kDa protein (D1) of reaction center of PS II (D1) is replaced [42]. Selective proteolysis is involved, inactive form of D1-protein is removed, and newly synthesized D1-polypeptide is integrated into the PS II holocomplex [41,43]. High rate of D1-protein turnover provides stability of thylakoid membranes and their electron-transport chain to damaging action of free radicals formed under stress conditions [30]. Thus, the literature and our results suggest that the biochemical response at the level of D1-turnover and intensive synthesis of polypeptides (60; 40.5 and 28–24.5 kDa) could act as a general adaptation signal for the plant in response to water stress.

In parallel we also measured the fluorescence emission spectra (77 K) of chloroplasts from normally irrigated and drought-stressed plants. As shown in Fig. 4, chloroplasts from drought-sensitive genotype Giymatli-2/17 have more intensive fluorescence at 740 nm from PS I under normal water supply. The F687/F740 ratio of control (non-drought stressed) chloroplasts of genotype Azamatli-95 was close to 0.38 and for genotype Giymatli-2/17-0.35. The shift of the main peak from 742 to 740 (in Azamatli-95) and from 740 to 738 nm (in Giymatli-2/17) is observed in both genotypes grown under water deficit. According to the data on the contents of pigments in leaves with normal irrigation and in the plants, subjected to water deficit, a short wavelength shift of the main maximum in the fluorescence spectra is coupled with a decrease in the amount of chlorophyll in PS I antenna (Fig. 2). The fluorescence intensity at 740 also slightly increased. 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. It is especially observed in drought-sensitive genotype 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 antenna system of the photosynthetic apparatus in the drought-tolerant genotype Azamatli-95 is rapidly reorganized, and plants began to adapt to environmental stress. More frequently changes in F687/740 ratio may be explained by redistribution of excitation light energy between PS II and PS I. At the same time, it seems realistic also to explain that this rise in short wave fluorescence

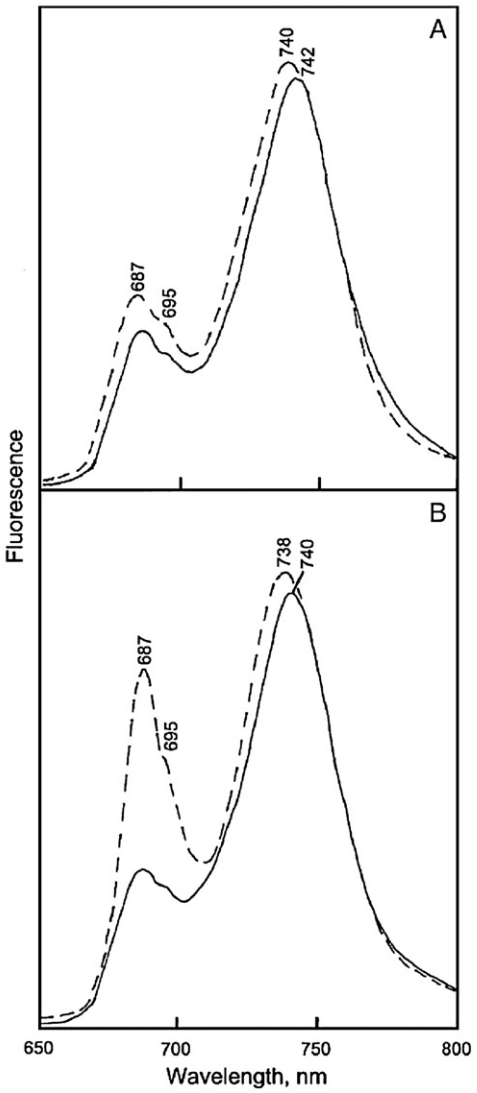

Fig. 4. Fluorescence emission spectra at 77 K of chloroplasts from drought-tolerant Azamatli-95 (A) and drought-sensitive Giymatli-2/17 (B) genotypes grown under normal water supply (solid curves) or drought conditions (dashed curves). Excitation wavelength 440 nm.

intensity is due to the lower content of RCs of PS II synthesized under water deficit.

Significant differences were found in functional activity of photosynthetic apparatus at the level of photochemical reactions of chloroplasts in comparative studies of genotypes distinguishing by architectonics and drought resistance. In our experiments, the highest PS II activity (oxygen evolution rate) of irrigated plants was found in drought-sensitive genotype Giymatli-2/17 with broad and drooping leaves (Table 1). Drought stress causes a significant change in the photochemical activity of chloroplasts in both genotypes. The electron transport activities of all stressed plants were lower than in the control plants. However, the activity of PS II was significantly affected by dehydration in cv. Giymatli-2/17—only 41% of control values remained. In drought-stressed cv. Azamatli-95 leaves the photochemical activity of PS II was about 78% of the control value. The case of PS II inactivation in

Table 1
The Photosystem II and Photosystem I activity in chloroplasts from wheat genotypes subjected to drought stress (μmol $O_2 \cdot mg^{-1}$ chlorophyll$\cdot h^{-1}$)

Genotypes	Photosystem II $H_2O \rightarrow K_3Fe(CN)_6$	in %	Photosystem I DCIP·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

both genotypes may be a suppression of synthesis of 32 kDa protein (D1-RC of PS II), which is a carrier of photochemical active forms of Chl a P680, or breach of electron transfer from pheophytin—intermediate electron carrier on quinone acceptor (Q_A) in non-cyclic transport of electrons. All else possible, 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 1). It can be caused by a higher ability of PS I to adapt to dehydration.

Concerning the side of drought stress action, several authors reported that PS II photochemistry is predisposed by drought stress to photoinhibitory damage [44,45]. In contradiction, Genty et al. [46] concluded that PS I-mediated electron transport was inhibited by drought, whereas PS II electron transport remained the same. During rehydration PS II activities recover slowly, but PS I complexes recovered their functional forms very quickly (within 1 min) [47].

So, dehydration involves many changes resulting in disrupted membrane integrity, suppression of many photosynthetic genes, decreased activities of photosystems, etc. 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. 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. 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 genotype Giymatli-2/17 responded to a period of stress by reducing photosynthetic efficiency and biomass accumulation. In this genotype the defense mechanisms prevent plants from suffering irreversible damages during drought. Therefore, in cv. Azamatli-95 the photosynthetic electron transport was probably sufficient to preclude the buildup of excess energy in PS II [7]. On the other hand, drought tolerant genotype Azamatli-95 seems able to avoid drought stress by maintaining a high photosynthetic activity, and does not suffer an oxidative stress high enough to trigger the defense mechanisms active in the genotype Giymatli-2/17.

Thus, in the drought-affected tolerant wheat the lower degree of changes in membrane, in comparison with the sensitive genotype, is probably due to the presence of a more fluid bilayer [48], since neither non-bilayer-forming lipids of thylakoid membrane nor free fatty acids (FFA) accumulated following drought. Changes in fatty acid saturation are required to preserve an appropriate balance of bilayer- and nonbilayer-forming lipids in the membrane. In drought adaptation, it is probably the occurrence of bilayer/nonbilayer transformations and their influence on the packaging of proteins that are of primary importance [16]. The larger capacity of the charged lipids to swell with water may also have increased the ability to bind water in the tolerant cultivar.

The obtained results may provide an entry point and a reference to future analysis of gene expression during drought. In addition, these results can suggest possible targets for the enhancement of stress tolerance in crops by genetic engineering. The data presented by us here might be used for monitoring environmental stresses in field-grown plants and help in selecting stress-resistant varieties for growth under unfavorable conditions.

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