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Protein Composition and Native State of Pigments of Thylakoid Membrane of Wheat Genotypes Differently Tolerant to Water Stress

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Abstract—Protein composition and native state of chlorophylls were analyzed in two wheat (*Triticum durum* L.) genotypes with different tolerance to drought, Barakatli-95 (drought-tolerant) and Garagylchyg-2 (drought-sensitive), during water deficit. It is shown that the plants subjected to water deficit appear to have a slight increase in α- and β-subunits of CF_1 ATP-synthase complex (57.5 and 55 kD, respectively) in Barakatli-95 and their lower content in Garagylchyg-2. Steady-state levels of the core antenna of PS II (CP47 and CP43) and light-harvesting Chl *a*/*b*-apoproteins (LHC) II in the 29.5-24 kD region remained more or less unchanged in both wheat genotypes. The synthesis of 36 kD protein and content of low-molecular-weight polypeptides (21.5, 16.5, and 14 kD) were noticeably increased in the tolerant genotype Barakatli-95. Drought caused significant changes in the carotenoid region of the spectrum (400-500 nm) in drought-sensitive genotype Garagylchyg-2 (especially in the content of pigments of the violaxanthin cycle). A shift of the main band from 740-742 to 738 nm is observed in the fluorescence spectra (77 K) of chloroplasts from both genotypes under water deficiency, and there is a stimulation of the ratio of fluorescence band intensity F687/F740.

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The responses of crop plants to abiotic stress are a serious problem and an important selective factor in evolution of plants, which influences their growth and development [1-4]. To cope with these external stresses, plants adopt numerous morphological, physiological, and metabolic responses [5]. Study of the mechanisms by which plants perceive and transform stressful signals is a key to genetic perfection of tolerance to stress through biotechnology. Detailed understanding of the molecular-genetic bases of resistance and mechanisms of stress adaptation of plants is necessary for answering this problem.

Among crop plants, durum wheat (*Triticum durum* L.), which is often grown in water-limited conditions, is an attractive study system because of the natural genetic variations in traits related to drought tolerance [6].

Of crucial importance in desiccation-tolerant plants are the properties of the photosynthetic apparatus, which is very sensitive and liable to injury and needs to be maintained or quickly repaired as soon as water enters again into the cells. In response to drought, the adaptation shown by many plants is directly connected with the membrane proteins, which respond to stress with significant qualitative and quantitative reorganizations. Proteins associated with sterols, carotenoids, and lipids define the molecular structure, physical and chemical properties, and functional activity of photosynthetic membranes. The function of membrane proteins are influenced by the lipid matrix in which they are embedded, and changes in the physical properties of bulk membrane lipids can induce changes in the structure and function of several protein complexes of the thylakoid membrane [7, 8]. Within the thylakoids, the membrane lipids play an important role in stabilizing the structural arrangement and, via the lipid–protein interactions, in integrating the protein complexes and possibly in maintaining their spatial distribution [9].

In the present work, investigations were carried out for more detailed understanding of the molecular mechanisms of the protectively adaptive processes directed to increase the resistance of the photosynthetic apparatus to

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drought. Two wheat genotypes with different tolerance to drought were either grown under normal water supply or subjected to water deficit. Detailed study of the influence of this process on synthesis and accumulation of chloroplast proteins and their spectral characteristics will reveal the possible role and importance of reorganizations of protein biosynthesis in development of tolerance and adaptation of plants to water stress.

MATERIALS AND METHODS

The objects of this study were two wheat genotypes (*Triticum durum* L.), Garagylchyg-2 (drought sensitive) and Barakatli-95 (drought tolerant). Plants were grown in plastic pots (20 cm diameter) containing a mixture of garden soil and sand (1 : 1 v/v) in a controlled environment. After two days of storage in darkness, the pots were transferred to light (photon flux density of 100 W/m^2 supplied by fluorescent LB-40 W lamps (Razno, Russia)). The day/night mode in the chamber was 16 h light at 25°C and 8 h dark at 20°C, 60 to 70% relative humidity. Parameters of water mode were determined according to a published method [10]. Starting from 10 days after sowing, when the height of plants was about 3 cm, drought stress was achieved by cutting water application. In one set, control plants from both genotypes were regularly watered, and another set of plants was subjected to water deficit by withholding water for 15 days. Plants were harvested 25 days after sowing (they were 15 to 20 cm high). Roots and shoots were separated. Leaves were taken for chloroplast isolation and various measurements.

Plants were mixed with chilled grinding buffer for chloroplast isolation containing of 0.4 M sucrose, 20 mM Tris, 10 mM NaCl, 1 mM EDTA (sodium salt), 0.1% polyethylene glycol, pH 7.8 at 4°C, and homogenized for 5 sec in a MPW-302 blender (Mechanika Precyzya, Poland). The homogenate was filtered through four layers of cheesecloth. Chloroplast isolation and thylakoid membrane precipitations were carried out according to a published method [11]. The chlorophyll concentration was determined spectrophotometrically in 80% acetone extract [12].

Thylakoid membrane proteins were analyzed according to Laemmli [13] using a 10 to 25% (w/v) linear gradient polyacrylamide gel in the presence of SDS as described earlier [11]. After electrophoresis, the gels were stained for 30 min (before boiling) with a solution of 0.04% Coomassie Brilliant Blue G-250 (France) prepared in 3.5% perchloric acid ($HClO_4$). The gels were scanned using an Ultroscan 2202 densitometer (LKB, Sweden) with a 633 nm laser as the light source. If necessary gels were dried in a special device (Slab Gel Dryer-2003, LKB). A set of standard proteins (kD) 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.

The measurements of fluorescence (F) and absorbance spectra (A) at 77 K were performed using a Hitachi-850 (Japan) fluorescence spectrophotometer and a double-beam Hitachi-557 (Japan) spectrophotometer as described previously [14]. Fluorescence emission spectra were corrected for the spectral sensitivity of the spectrofluorimeter using rhodamine B. The samples were immersed in liquid nitrogen during measurement.

RESULTS AND DISCUSSION

Figure 1 shows Coomassie blue staining SDS-PAGE analysis (a) and density patterns (b) of thylakoid membrane proteins of wheat genotypes with different tolerance to drought. Thylakoid membranes isolated from the wheat genotypes contained about 23 polypeptides with molecular masses from 14 to 112 kD. Comparative analysis of samples grown under normal water supply and the samples subjected to a drought presented the following picture. The content of photosystem (PS) I core (CP I, 112 kD) and apoprotein of P700 (68 kD) increases in the tolerant genotype Barakatli-95 compared to non-stressed seedlings. However, the intensity of these polypeptides slightly decreases in the drought-sensitive genotype Garagylchyg-2. The plants subjected to water deficit appeared to have a slight increase in α- and β-subunits of CF_1 ATP synthase complex (57.5 and 55 kD, respectively) in Barakatli-95 and their lower content in Garagylchyg-2. The low content of β-subunits of CF_1 ATP synthase complex has been also shown in pea plants subjected to drought at high light exposure [15]. Steady-state levels of the core antenna of PS II (CP47 and CP43), serving as the connecting antenna between the main light harvesting complex LHC II and reaction center of PS II, and of light-harvesting Chl *a*/*b*-apoproteins in the 29.5-24 kD region remained more or less unchanged in both cultivars. These results agree with data that were obtained early [15, 16]. Thylakoid membranes from Garagylchyg-2, on the other hand, showed reduced level of 34 and 32 kD protein synthesis.

It is revealed that 36 kD polypeptide noticeably increased in the tolerant genotype Barakatli-95. It has been assumed that this polypeptide band is associated only with high levels of tolerance [17]. At the same time, increase in 21.5 kD polypeptide is also observed in both genotypes under drought. It is interesting to note that the content of this polypeptide especially strongly increases (about 2-fold higher) in the drought-resistant genotype Barakatli-95. Recent studies on the adaptation of plants to osmotic stress revealed the participation of drought-stress induced proteins with molecular masses of 20-22 kD possessing a sequence similarity with class II WSCPs (water-

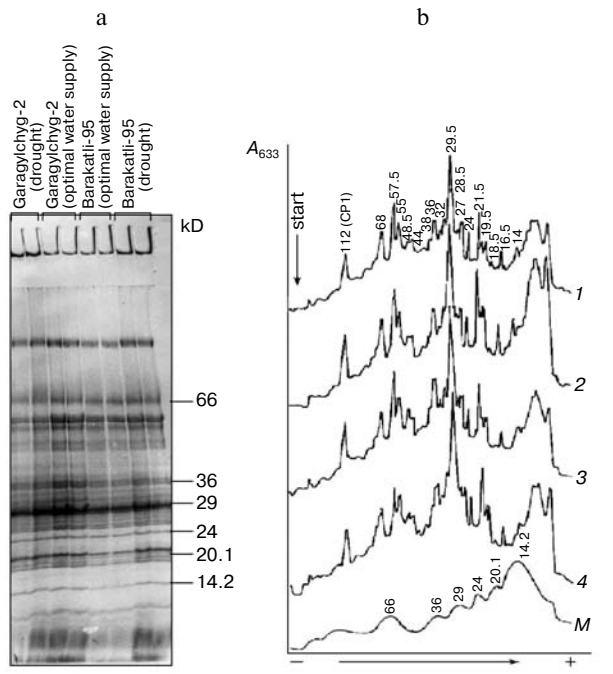

Fig. 1. Coomassie blue staining from SDS-PAGE (10-25% gel) analysis (a) and density patterns (b) of thylakoid membrane proteins of chloroplasts from wheat plants grown under optimal water supply (Barakatli-95 (*1*) and Garagylchyg-2 (*3*)) and drought conditions (Barakatli-95 (*2*) and Garagylchyg-2 (*4*)). M, standard proteins (kD): bovine serum albumin (66), glyceraldehyde-3-phosphate dehydrogenase (36), carbonic anhydrase (29), trypsinogen (24), trypsin inhibitor (20.1), and α-lactalbumin (14.2). Samples in different quantities were placed on the gel.

soluble chlorophyll (Chl) proteins) [18]. The WSCP is a tetramer, which may have only one or no Chl molecules in each subunit [19]. Expression of WSCP is induced by drought [20] and heat stresses [21]. It is supposed that this protein might be involved in the decrease in the protease activity in the drought-adapted leaves, thus contributing to delay in leaf senescence. There are data in the literature indicating that it also is induced by salt stress.

Drought also caused an increase in the synthesis of low-molecular-weight polypeptides (16.5 and 14 kD) in the tolerant genotype Barakatli-95, which can promote stabilization of functional activity of chloroplasts in conditions of water deficit. However, such effects are not observed in the drought-sensitive genotype Garagylchyg-2. It is possible to assume that these proteins are related to ELIP (early light-inducible proteins), a recently described class of carotenoid-binding proteins that are directly synthesized on transfer of etiolated plants to light or after photoinhibition [22, 23]. It has also been shown that they are present (though in low quantity) in green leaves [24]. The carotenoid/Chl *a*+*b* ratio increases in plants subjected to drought, indicating a protective role of these polypeptides under drought. Correlation between tolerance and increase in synthesis of the some low-molecular-weight polypeptides including those of 36, 21.5, 16.5, and 14 kD assumes that changes in expression of genes of these polypeptides 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 kD protein (D1) of reaction center of PS II is replaced [25]. Selective proteolysis is involved, inactive form of D1-protein is removed, and newly synthesized D1-polypeptide is integrated into the PS II holocomplex [26-28]. 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. Thus, the literature and our results suggest that the biochemical response at the level of D1 turnover and intensive synthesis of low-molecular-weight polypeptides (36, 21.5, 16.5, and 14 kD) could act as a general adaptation signal for the plant in response to water stress.

Simultaneously, spectral characteristics of the wheat genotypes subjected to drought were registered to track the state of native forms of pigments. Low-temperature fluorescence spectra (77 K) of chloroplasts from the control and drought-stressed wheat seedlings are shown in Figs. 2 and 3. The shift of the main peak from 742 to 738 nm (in Barakatli-95) and from 740 to 738 nm (in Garagylchyg-2) is observed in both genotypes grown under water deficit. According to the data on the contents of pigments in seedlings with normal water supply and in

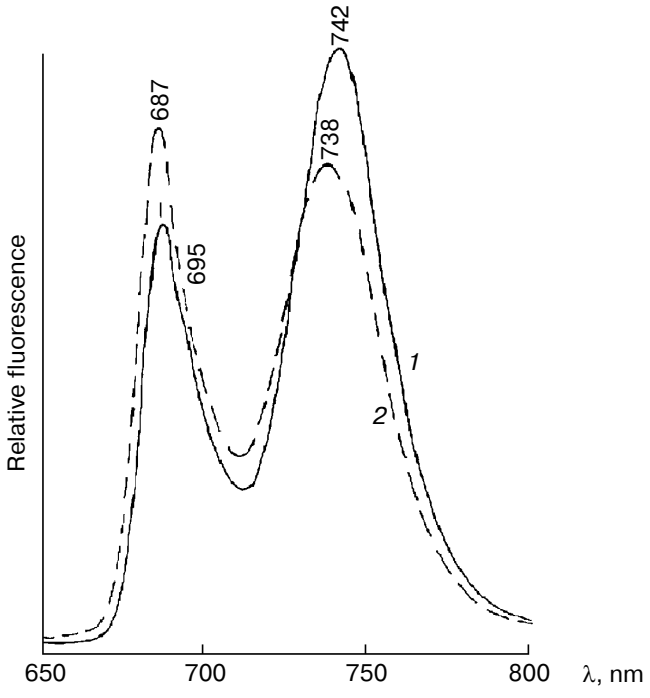

Fig. 2. Fluorescence emission spectra at 77 K of chloroplasts from genotype Barakatli-95 grown under optimal water supply (*1*) or drought conditions (*2*).

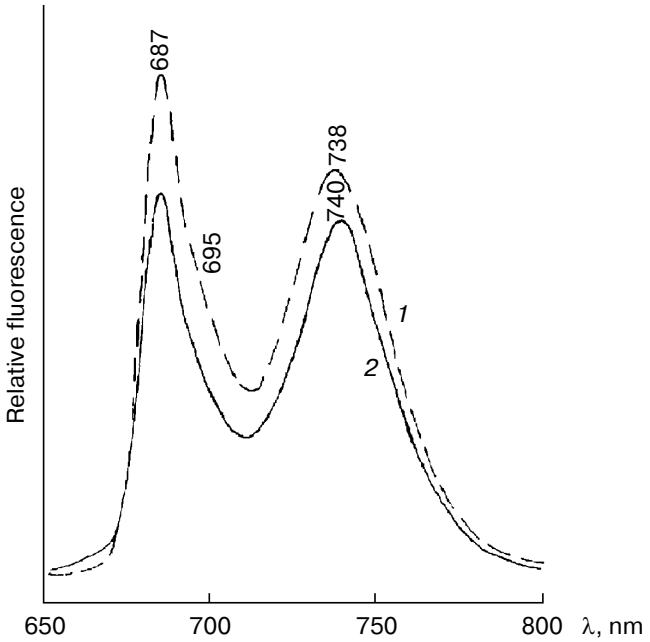

Fig. 3. Fluorescence emission spectra at 77 K of chloroplasts from genotype Garagylchyg-2 grown under optimal water supply (*1*) or drought conditions (*2*).

the plants subjected to stress, a short wavelength shift of the main maximum in the fluorescence spectrum is coupled with a decrease in the amount of chlorophyll in PS I antenna. Short wavelength peaks at 687 and 695 nm corresponding to core antenna of PS II (CP43 and CP47) remained in both cultivars after drought, and the intensity of these peaks considerably increased. At the same time, increase in the ratio of fluorescence bands F687/F740 takes place. In the genotype Barakatli-95, this value is 0.71 and 1.05 under normal water supply and under stress, and it is 1.15 and 1.25 in genotype Garagylchyg-2, respectively. The results suggest that antenna system of the photosynthetic apparatus in the drought-tolerant genotype Barakatli-95 is rapidly reorganized, and plants began to adapt to environmental stress. It is possible that antenna pigments of the plants much more move from the reaction centers into the lipid bilayer of thylakoid membranes for display of the protective mechanism of plants on survival under water deficit conditions.

Absorption spectra (A) and their fourth derivatives (A^{IV}) of chloroplasts isolated from wheat seedlings subjected to drought were also measured. As shown in Fig. 4, changes in the content and localization of native forms of pigments in Barakatli-95 genotype are not observed. However, in case of Garagylchyg-2 there is reorganization

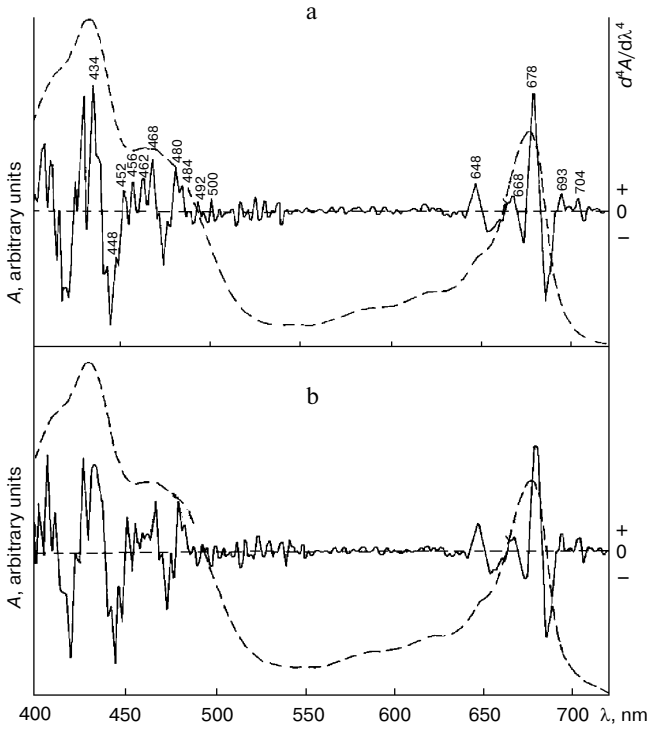

Fig. 4. Absorption spectra (dashed curves) and fourth derivatives of absorption spectra (solid curves) at 296 K of chloroplasts from genotype Barakatli-95 grown under normal water supply (a) or water deficit conditions (b).

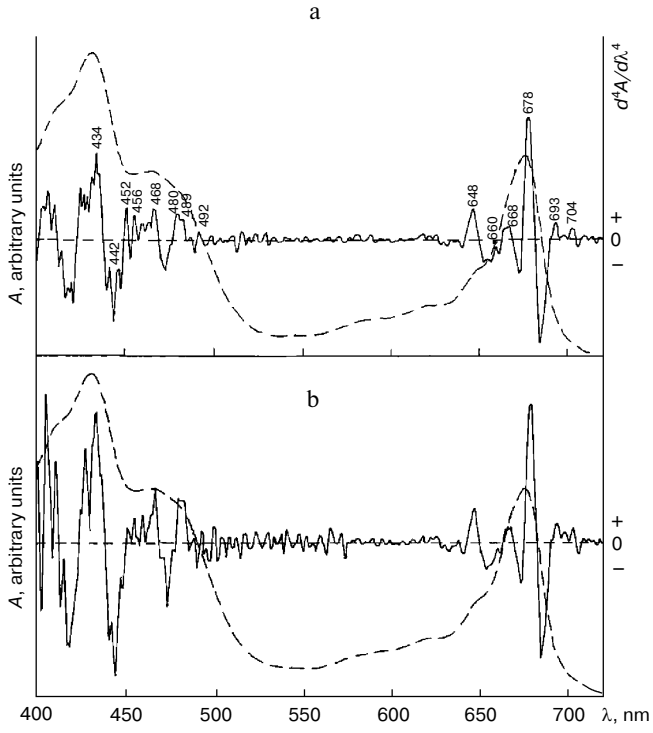

Fig. 5. Absorption spectra (dashed curves) and fourth derivatives of absorption spectra (solid curves) at 296 K of chloroplasts from genotype Garagylchyg-2 grown under normal water supply (a) or water deficit conditions (b).

of carotenoid region bands (400-500 nm) of the spectrum (especially in the content of pigments of the violaxanthin cycle): 420, 430, 434, 448, 468, 472, 480, 487, 492, and 500 nm (Fig. 5). In the tolerant genotype these changes are not observed, which may be explained by its higher total rate of electron transport to preclude the buildup of excess energy in PS II [29]. On the other hand, drought-tolerant genotype Barakatli-95 seems able to avoid drought stress by maintaining a high photosynthetic activity, and it does not suffer oxidative stress high enough to trigger the defense mechanisms active in the genotype Garagylchyg-2. Consequently, under drought it can maintain a growth rate similar to that of well-watered control plants.

So, for drought-tolerant genotypes low level of changes in spectral characteristics in comparison with sensitive genotype was probably due to the presence of more lipids in the lipid bilayer [7]. Changes in fatty acid saturation are required to preserve an appropriate balance of bilayer- and monolayer-forming lipids in the membrane. In drought adaptation, it is probably the occurrence of bilayer/monolayer transformations and their influence on the packaging of proteins that are of primary importance. Neither non-bilayer-forming lipids of thylakoid membrane nor free fatty acids accumulated following stress.

The presented data indicates that drought probably changes the rate of synthesis of particular polypeptides of the photosynthetic membrane, and therefore a redistribution of their relative part in groups with different molecular mass is observed. The results obtained in this study suggest considerable reorganization in the photosynthetic apparatus of wheat during adaptation to stress conditions and can be useful in the further search of genes and groups of the genes responsible for resistance of the given plant. Thus, it suggests that the changes observed in protein composition and pigment organization of the photosynthetic apparatus may compose a basis for molecular protectively adaptive processes directed to increase in wheat tolerance to drought.

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