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Response of Photosynthetic Apparatus and Antioxidant Defense Systems in *Triticum aestivum* L. Genotypes Subjected to Drought Stress

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Two wheat *(Triticum aestivum* L.) genotypes contrasting in architectonics and differing in drought-resistance, Azamatli-95 (short-stemmed, with vertically oriented small leaves, drought-tolerant) and Giymatli-2/17 (short-stemmed, with broad and drooping leaves, drought-sensitive) were grown in field conditions in a wide area under normal water supply and severe water deficit. It was found out that the content of CPI (M_r 115 kDa), apoprotein of P_{700} with M_r 63 kDa and LHCII polypeptides insignificantly increases in the drought-resistant Azamatli-95 under extreme water supply condition while their content decreases in drought-sensitive 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 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 Giymatli-2/17 shows a larger reduction in the actual PSII photochemical efficiency of chloroplasts than Azamatli-95. The activitites of antioxidant enzymes such as catalase, peroxidase, glutathione reductase and superoxide dismutase differently change in wheat genotypes during ontogenesis.

Keywords: *drought, reactive oxygen species, relative water content, photosystem, chlorophyll, sodium dodesyl sulphate, fluorescence, activity, antioxidant enzymes, wheat genotypes*

INTRODUCTION

Plants are subjected to a range of abiotic and biotic stresses that affect their growth and development. In particular, it is predicted that water deficit will continue to be a major abiotic stress factor affecting global crop yields (Sharma and Lavanya, 2002). One third of the world's population resides in water-deficient regions, and with elevated CO_2 levels in the atmosphere and climatic changes predicted in the future, drought could become more frequent and severe.

Wheat is one of the widely cultivated crops in Azerbaijan, where drought is the main abiotic stress limiting its grain yield (Aliev, 2001; Aliyev, 2002).

In response to stress, plants activate a number of defense mechanisms that function to increase tolerance to adverse conditions. The response to drought stress which involves a number of biochemical-molecular mechanisms is complex. The application of this emerging understanding to the genetic engineering of food crops has already led to examples of improved drought tolerance and increased yield under drought (Hu et al., 2008).

Production of reactive oxygen species (ROS) and other radicals increases dramatically during water deficiency, and enhanced levels of reactive oxygen species are generated in various intracellular compartments in plants and may cause oxidative damage or act as signals (Gechev et al., 2006). The enhanced production of ROS in chloroplasts and peroxisomes has been correlated with drastic changes in nuclear gene expression that reveals the transfer of $^1O_2$-derived signals from the plastid to the nucleus. Many of the $^1O_2$-responsive genes are different from those activated by superoxide ($O_2^{\bullet-}$) or H_2O_2, suggesting that $O_2^{\bullet-}$/H_2O_2- and $^1O_2$-dependent signaling occurs via distinct pathways. These pathways could act independently or may interact with each other (Baruah et al., 2009). Plants protect cellular and subcellular system from the cytotoxic effects of active oxygen radicals with antioxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and glutathion reductase (GR) as well as metabolites like glutathione, ascorbic acid, α-tocopherol and carotenoids.

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. 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 (Friso et al., 2004).

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The aim of this study was to investigate structural and functional characteristics of thylakoid membrane and antioxidant enzymes that ensure 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 developing drought tolerant varieties.

MATERIALS AND METHODS

In the experiments we used two bread wheat genotypes (*Triticum aestivum* L.), contrasting in architectonics and differing in drought resistance, Giymatli-2/17 – short-stemmed, with broad and drooping leaves and grain yield of 7-8 t ha^{-1}, drought-sensitive; Azamatli-95 – short-stemmed, with vertically oriented small leaves and grain yield of 8-9 t ha^{-1}, drought-tolerant. All genotypes were grown in field conditions in the wide area under normal water supply and dryland conditions. The plants were provided by Experimental Station of the Research Institute of Crop Husbandry (Baku, Azerbaijan). Different sensitivities of these genotypes to drought were determined during some years in different regions of Azerbaijan based on grain yield (Aliev, 1998, 2001). A group of plants from both genotypes 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.

Leaf relative water content (RWC) was estimated gravimetrically according to Tambussi et al. (2005).

Leaves were homogenized with a Waring blender 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, 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. (1992).

The chlorophyll (Chl) concentration was determined in 80% acetone extract (Mc-Kinney, 1941). Samples were frozen in liquid nitrogen and stored at -80°C until required.

For polypeptide analysis samples of thylakoid membranes were separated under denaturing conditions at 2° to 3°C in the presence of 0.1% (w/v) SDS (sodium dodesyl sulphate) using a 10 to 25% (w/v) linear gradient polyacrylamide gel (acrylamide:methylenebisacrylamide ratio = 30/0.8) in combination with the Laemmli buffer system (Laemmli, 1970) as described previously (Guseynova et al., 2001). 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 (Sigma, USA) was used for the determination of the molecular masses of polypeptides.

The measurements of fluorescence (F) at 77 K were performed using a Hitachi-850 (Japan) fluorescence spectrophotometer as reported previously (Asadov et al., 1986). Fluorescence emission spectra were corrected for the spectral sensitivity of the spectrophotometer using rhodamine B. Chlorophyll fluorescence was excited by dark blue light with wavelength of 440 nm. The samples on quartz glass fiber were quickly frozen at 77 K by dipping the glass fiber into liquid nitrogen.

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 μE $m^{-2}s^{-1}$), according to Guseynova et al. (2006). Chloroplasts concentrations equivalent to 100 μg Chl were used for all measurements.

Measurements of photoinduced changes of fluorescence yield from F_o level to F_{max} were carried out at room temperature using laboratory-built set-up as described earlier (Klimov et al., 1982). Potential quantum yield of photosystem (PS) II was estimated according to the formula:

$$\Phi_p = F_v/F_m = (F_m - F_o)/F_m$$

Enzyme extract was prepared by homogenizing leaf material (1 g fr wt) with a pestle in an ice-cold mortar with Na_2HPO_4/NaH_2PO_4 buffer. The homogenates were filtered through four layers of cheesecloth and then centrifuged at 4°C. The supernatant were collected and used for the assays of enzymatic activities.

The activity of catalase was determined as a decrease in absorbance at 240 nm for 1 min following the decomposition of H_2O_2 as described by Kumar and Knowles (1993). The reaction mixture contained 50 mM phosphate buffer (pH 7.0) and

15 mM H_2O_2, and reaction was initiated by adding enzyme extract.

The activity of ascorbat peroxidase (APO) was assayed according to Nakano and Asada (1981). The assay mixture consisted of 0.05 mM ASA, 0.1 mM H_2O_2, 0.1 mM EDTA, 50 mM sodium phosphate buffer (pH 7.6), and 0.3 mL enzyme extract. The activity was measured as a decrease in absorbance at 290 nm for 30 sec.

Glutathione reductase activity was determined at 340 nm for 10 min in reaction mixture containing 100 mM potassium phosphate buffer (pH 7.8), 1 mM EDTA, 0.2 mM NADPH and 0.5 mM GSSG (Yannarelli et al., 2007).

Superoxide dismutase activity was estimated by using SOD Assay Kit-WST (Sigma-Aldrich, USA). The absorbance was recorded at 450 nm and one enzyme unit of SOD activity was defined as the amount of enzime recuired to cause 50% inhibition of the rate of NBT reduction.

Protein concentration was determined according to Sedmak and Grossberg (1977) by using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

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 those subjected to water stress. 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 Giymatli-2/17 than in the more tolerant 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 Azamatli-95, even though both genotypes showed a certain drop in RWC.

A reduction in the total chlorophyll content and Chl *a/b* ratio occurred during drought stress. 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 Giymatli-2/17. A drought-induced decrease in pigment contents was previously reported in several plant species, including pea (Moran et al., 1994), durum wheat (Loggini et al., 1999) and *Boea hydroscopica* (Navari-Izzo et al., 2000). The more drought-sensitive Giymatli-2/17 showed a slight increase in the pool size of xantophyll-cycle components, but such effect was not shown in the tolerant Azamatli-95, that may be explained by its higher rate of electron transport compared with Giymatli-2/17 (Guseynova et al., 2006).

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. In addition, the photosynthetic apparatus may show acclimation responses such as changes in the relative proportion of stacked and unstacked membrane domains (Anderson and Aro, 1994). 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. Another influential factor might be the rise due to water loss in the stroma ionic charge screening the repulsive force between thylakoids (Barber, 1982).

The protein profiles of thylakoid membranes in non-stressed and water-stressed plants were analysed. Figure 1 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 Figure 1, thylakoid membranes isolated from the wheat genotypes grown under normal water supply appeared to have about 26 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 PSI core (CPI) 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 CPI (115 kDa) and apoprotein of P_{700} (63 kDa) were maintained at relatively high levels in tolerant genotype Azamatli-95, but were slightly little affected by drought in more sensitive genotype 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 Azamatli-95. However, a detection of this polypeptide was not available in the experiments with

seedlings of wheat grown in growth chamber under controlled environmental conditions (Guseynova et al., 2006). On the basis of obtained results 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 (Wisnewski et al., 1999). The dehydrin family of proteins is induced by environmental stresses that result in cellular dehydration (Close, 1997). 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-subunits 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 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 (Giardi et al., 1995; Guseynova et al., 2006). Steady-state levels of the core antenna of PSII (CP 47 and CP 43) serve as the connecting antenna between the main light harvesting complex LHCII and reaction center of PSII remained more or less unchanged in both genotypes. These results agree with data that were obtained earlier (Giardi et al., 1995; Guseynova et al., 2006).

The most striking change was a reveal of protein with molecular mass of 40.5 kDa in tolerant genotype Azamatli-95. It is absent in leaves from non-stressed plants, but at a lower level was detected only in tolerant genotypes subjected to water deficit. According to the current literature, C 40.4 protein share high sequence homology with 34 kDa thylakoid protein CDSP (chloroplastic drought-induced stress protein), previously described 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 (Gillet et al., 1998). The accumulation of two chloroplastic nuclear-encoded proteins in water-stressed *Solanum tuberosum* plants were reported (Pruvot et al., 1996). A stromal protein of 32 kDa related to thioredoxins was suggested to maintain the redox state of chloroplastic proteins upon drought stress (Rey et al., 1998). 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 C 40.4 protein is closely bounded with LHCII and has functional role by modeling photosynthetic effectiveness and light dissipation of excess absorbed light energy inside antenna complex (Monte et al., 1999).

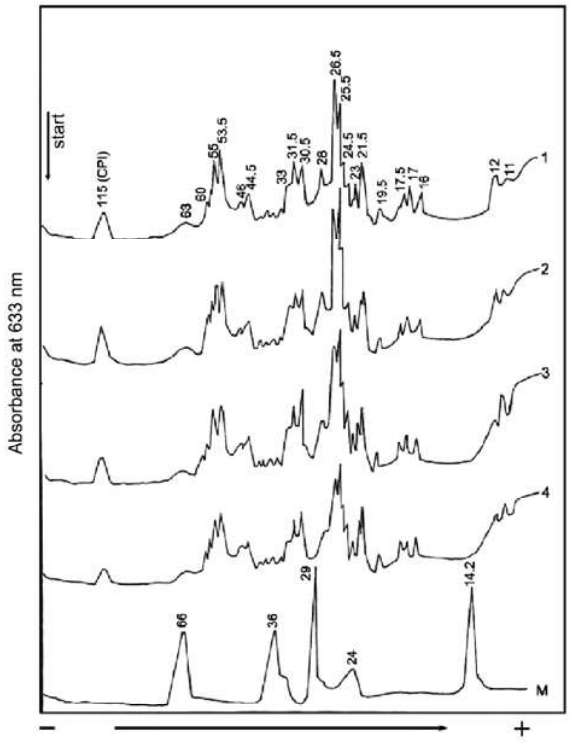

Figure 1. Density patterns from Coomassie blue staining SDS-PAGE (10-25% gel) analysis of thylakoid membrane proteins from wheat plants grown in field conditions under normal water supply (Azamatli-95) (1) and Giymatli-2/17 (3) and drought stress (Azamatli-95) (2) and Giymatli-2/17 (4). M, standard proteins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

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 Giymatli-2/17) seems to be in part due to its enhanced degradation rate (Sippola et al., 1998; Guseynova et al., 2006). 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 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 relates 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 (Guseynova et al., 2006). It is supposed that this protein might be involved in 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 Azamatli-95, but significantly decreased in 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 PSII reparation during which the most damaged 32 kDa protein (D1) of reaction center of PSII is replaced (Melis, 1998). Selective proteolysis is involved, inactive form of D1-protein is removed, and newly synthesized D1-polypeptide is integrated into the PSII holocomplex (Chaloub et al., 2003). 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 (Guseynova et al., 2006). Thus, the literature and our results suggest that 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. Table 1 represents the data of membrane proteins in which quantitative changes are significant under drought stress.

In parallel we also measured the fluorescence emission spectra (77 K) of chloroplasts from normally irrigated and drought-stressed plants. As shown in Figure 2, chloroplasts from drought-sensitive genotype Giymatli-2/17 have more intensive fluorescence at 740 nm from PSI 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 pigments content 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 PSI antenna (Figure 2). The fluorescence intensity at 740 also slightly increased. The short wavelength peaks at 687 and 695 nm (fluorescence from the PSII 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

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

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

* Comment: (+) – protein content is increasing, (-) – content is decreasing, 0 – no changes

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 PSII and PSI.

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 PSII activity (oxygen evolution rate) of irrigated plants was found in drought-sensitive genotype Giymatli-2/17 with broad and drooping leaves (Table 2). 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 PSII was significantly affected by dehydration in Giymatli-2/17, only 41% of control values remained. In drought-stressed Azamatli-95 leaves the photochemical activity of PSII was about 78% of the control value. The case of PSII inactivation in both genotypes may be suppression of synthesis of 32 kDa protein (D1-protein of reaction centre (RC) of PSII), which is carrier of photochemical active forms of Chl a P_{680}, 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 PSII core complexes.

PSI activity (O_2 uptake rate), however, was affected much less under drought stress (Table 2). It can be caused by a higher ability of PSI to adapt to dehydration.

Concerning the side of drought stress action several authors reported that PSII photochemistry is predisposed by drought stress to photoinhibitory damage (Peltier et al., 1995). In contradiction, Genty et al. (1987) concluded that PSI-mediated electron transport was inhibited by drought, whereas PSII electron transport remained the same. During rehydration PSII activities recover slowly, but PSI complexes recovered their functional forms very quickly (within 1 min) (Hirai et al., 2004).

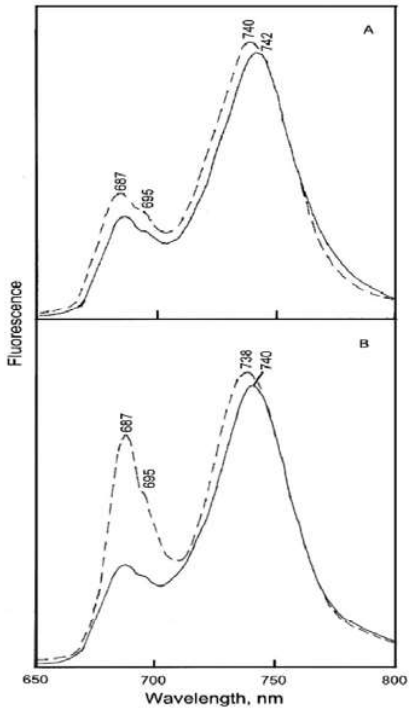

Figure 2. Fluorescence emission spectra at 77 K of chloroplasts from drought-tolerant Azamatli-95 (A) and drought-sensitive Giymatli-2/17 (B) genotypes grown under normal water supply (solid curves) or drought conditions (dashed curves).

It is known that fluorescence yield is minimal (F_o), when primary electron acceptor of PSII, plastoquinone (Q_A), is oxidized. Reduction of Q_A results in rise of chlorophyll fluorescence approximately 3-5 times up to F_m level. Rise of chlorophyll fluorescence yield from initial (F_o) to maximal (F_m) level, i.e. appearance of variable fluorescence (F_v, where $F_v=F_m-F_o$), reflects process of accumulation of reaction centers of PSII in "closed" state with reduced primary quinone acceptor (Q_A).

Values of fluorescent parameters that characterize the functional state of photosynthetic apparatus of winter wheat plants grown under different conditions of water regime are shown in Table 3. Potential quantum yield of photochemical reac tions of PSII (F_v/F_m ratio) in chloroplasts from control (non-drought stressed) plants was 0.74 for

Table 2. The Photosystem II and Photosystem I activity in chloroplasts from wheat genotypes subjected to drought stress (μmol O_2 mg^{-1} chlorophyll 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

Azamatli-95 and 0.81 for Giymatli-2/17, that is typical for normally grown plants. As it seems from Table 3 state of PSII in dehydration process was being significantly changed. Potential yield of photochemical reactions of PSII 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 one 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 value of photochemical efficiency of PSII under regular irrigation conditions of growing. However, low ratio of F_v/F_m again confirms that strong effect of drought is appeared in genotype Giymatli-2/17 (genotype Giymatli-2/17 is strongly affected by drought). Decreasing of a photochemical efficiency (F_v/F_m) under severe drought can be considered as a fact of damage of photosynthetic reaction centers.

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

Variant	Control	Drought	% from control
Azamatli-95			
F_o	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_o	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

*Comment: average arithmetic and standard mistakes from three independent experiments, each of which was carried out in double biological frequency, are shown in the table.

Both Q_B-reducing and Q_B-non-reducing complexes of PSII make a contribution in variable fluorescence (F_v). Charge separation is realized in Q_B-non-reducing complexes of PSII, but electrons are not transported to plastoquinone pool. Q_B-reducing complexes of PSII in active state are able to realize electron transport between Q_A and Q_B. They lose this ability when D1-protein is damaged and turn to Q_B-non-reducing complexes (Pshibytko et al., 2003). In optimal conditions due to reactions of reparation cycle the constant ratio between these types of complexes of PSII is supported. Probably, dehydration induces disruption of reactions at the acceptor side of PSII, expressed in increasing of a number of Q_B-non-reducing centers.

Under water deficit linear electron transport is suppressed by accumulation of plastoquinones caused from difficulty of lateral diffusion of plastoquinones because of increased viscosity of lipid bilayer (Hirai et al., 2004), that could be caused by increasing of reduction level of plastoquinone pool, damage of Q_B-binding site with D1-protein and deterioration of conditions of damaged D1-protein reparation (Melis, 1998).

Activity of antioxidative enzymes differed in investigated wheat genotypes under normal water supply condition at all stages of ontogenesis. Catalase (CAT) activity increased in both genotypes under water deficiency. Maximum level of catalase activity was revealed at the end of flowering in response to drought stress (Figure 3). As it is shown from the Figure 3, catalase activity was higher in Azamatli-95 than the corresponding control at all the stages of study. Nevertheless, activity of this enzyme was lower in two stages – end of earing and flowering in drought-sensitive genotype Giymatli-2/17 than the control. The increase of CAT activity in plants under water stress has been reported in other study (Quartacci and Navari-Izzo, 1992).

It is known that catalase reacts with H_2O_2 directly to form water and oxygen. The decrease in CAT activity in the end of ontogenesis could indicate its inactivation by the accumulated hydrogen peroxide by water shortage and could be explained partly by photoinactivation of the enzyme. When plants are not exposed to water stress, resynthesis of CAT compensates for the loss of total activity caused by irradiance. Inhibition of protein synthesis induced by water stress conceivably could impair resynthesis and partly account for the marked decrease in CAT activity in plants subjected to water stress in the light.

Ascorbate peroxidase (APO) activity in Giymatli-2/17 was higher under water deficiency than the corresponding control at all stages of development. In Azamatli-95 higher level of APO activity was revealed at the end of earing and at flowering stage, and in a stage of milky ripeness (Figure 4). Detailed study of APO activity dynamics in wheat plants grown under drought conditions allows to conclude that the function of APO increases during water deficiency. An increase of peroxidase (POD) activity was also observed by different authors during a drought and salt stress (Siegel, 1993). It indicates the formation of large amounts of H_2O_2 during water stress. Increases of activity can explain with some assumptions: elevated H_2O_2 concentrations could release POD from membrane structures, with which it is normally associated. POD could be synthesized *de novo* at least in some cases. Water stress could increase the accumulation of POD substrates, such as glutathione, ascorbate, and phenolic compounds, which, in turn, are scavengers of activated oxygen species.

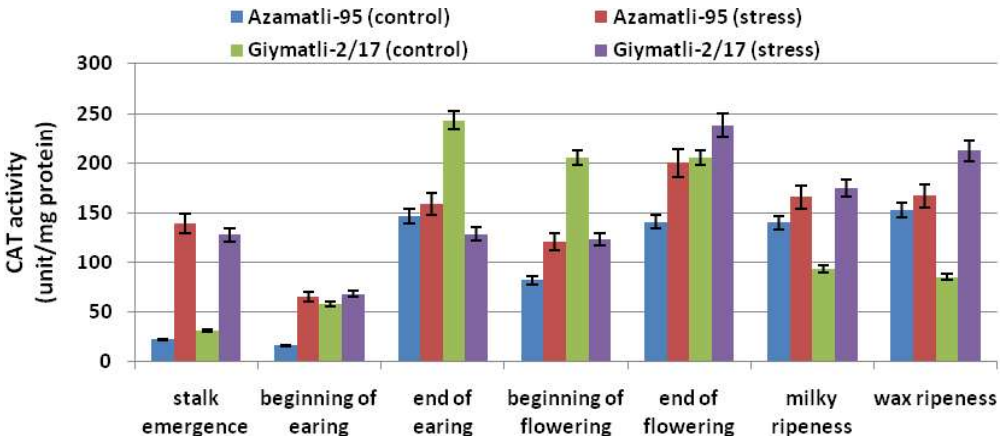

Figure 3. Effect of water stress on catalase activity in leaves from wheat genotypes at different stages of ontogenesis.

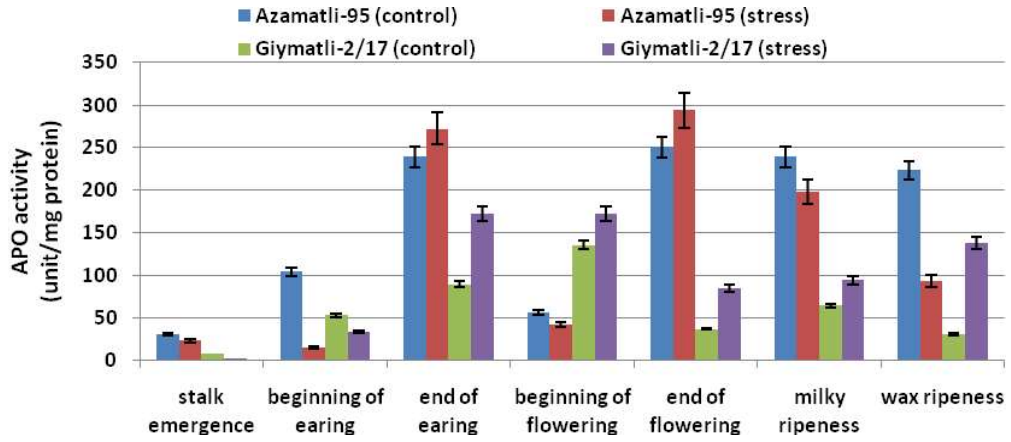

Figure 4. Effect of water stress on ascorbate peroxidase activity in leaves from wheat genotypes at different stages of ontogenesis.

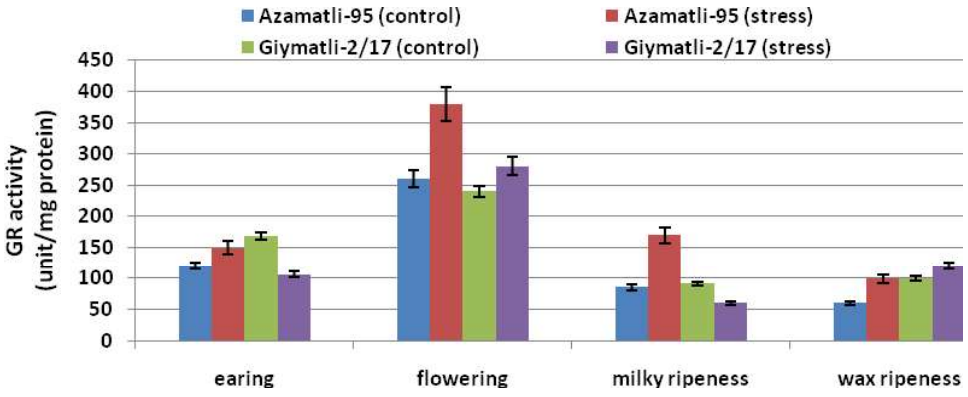

Figure 5. Effect of water stress on glutathione reductase activity in leaves from wheat genotypes at different stages of ontogenesis.

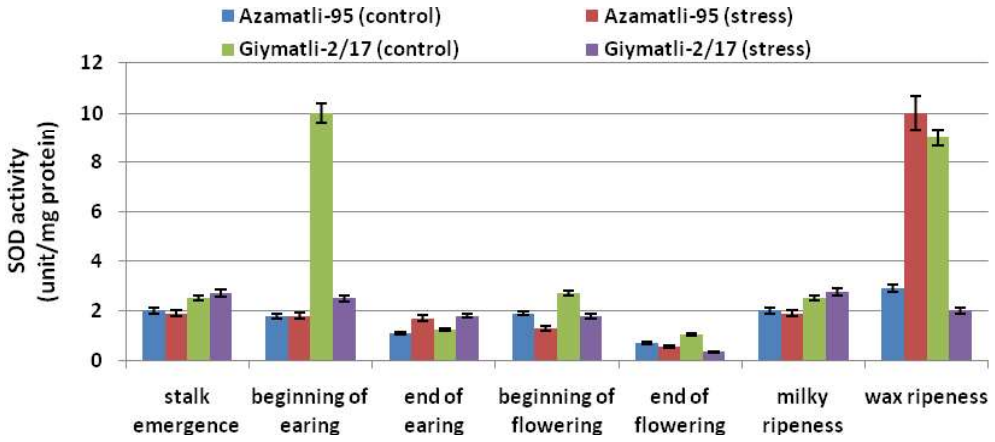

Figure 6. Effect of water stress on superoxide dismutase activity in leaves from wheat genotypes at different stages of ontogenesis.

Also it is known that H_2O_2 participates in signal transduction during development of oxidizing stress, inducing genes of cytosolic POD.

The maximum activity of glutathione reductase (GR) both as in the control, as well as in drought-subjected plants was observed at the flowering stage (Figure 5). In tolerant genotype Azamatli-95 in all the stages of ontogenesis GR activity was higher in the stress conditions compared with the control. In sensitive genotype Giymatli-2/17 the increase of activity was observed only in the stages of flowering and wax ripeness.

Dynamics of superoxide dismutase (SOD) functioning differed from other enzymes. SOD activity was lower in both genotypes as compared with control variants (Figure 6). Only in the end of ontogenesis, at wax ripeness stage, when drought effect is greatest, SOD activity increased in tolerant genotype Azamatli-95. Many authors specify key role SOD in antioxidative protection (Alscher et al., 2002). However, results obtained by us on SOD activity differed partly from the literary data. Nevertheless, it is known that plant cells contain a little izoform of SOD which probably unequally react to water deficiency. The study of these SOD isoforms during water stress induction revealed differential regulation of their activities: MnSOD and FeSOD activities increased rapidly while Cu/ZnSOD activities decreased in cowpea plants (Brou et al., 2007).

Obtained results suggest that water stress alters the equilibrium between free radicals production and enzymatic defense reactions in wheat genotypes and Giymatli-2/17 has less efficient antioxidant systems than 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 Azamatli-95 the photosynthetic electron transport was probably sufficient to preclude the build-up of excess energy in PSII (Loggini et al., 1999). 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. Dynamics of changes of antioxidative enzymes activity in wheat genotypes under normal water supply and deficiency of water in all stages of ontogenesis reveal that drought differently changes a balance between production of free radicals and enzyme reactions of protection.

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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