Biochimica et Biophysica Acta 1817 (2012) 1516–1523

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Photosynthetic characteristics and enzymatic antioxidant capacity of leaves from wheat cultivars exposed to drought ☆

Irada M. Huseynova *

Institute of Botany, Azerbaijan National Academy of Sciences, 40 Badamdar Shosse, Baku AZ 1073, Azerbaijan

ARTICLE INFO

Article history:
Received 11 November 2011
Received in revised form 28 February 2012
Accepted 29 February 2012
Available online 7 March 2012

Keywords:
Wheat cultivar
Chlorophyll fluorescence
Photosystem II
Antioxidant enzyme
Ontogenesis
Drought

ABSTRACT

Two durum *(Triticum durum* L.), Barakatli-95 and Garagylchyg-2; and two bread (*Triticum aestivum* L.) wheat cultivars, Azamatli-95 and Giymatli-2/17 with different sensitivities to drought were grown in the field on a wide area under normal irrigation and severe water deficit. Drought caused a more pronounced inhibition in photosynthetic parameters in the more sensitive cvs Garagylchyg-2 and Giymatli-2/17 compared with the tolerant cvs Barakatli-95 and Azamatli-95. Upon dehydration, a decline in total chlorophyll and relative water content was evident in all cultivars, especially in later periods of ontogenesis. Potential quantum yield of PS II (F_v/F_m ratio) in cv Azamatli-95 was maximal during stalk emergency stage at the beginning of drought. This parameter increased in cv Garagylchyg-2, while in tolerant cultivar Barakatli-95 significant changes were not observed. Contrary to other wheat genotypes in Giymatli-2/17 drought caused a decrease in PS II quantum yield. Drought-tolerant cultivars showed a significant increase in CAT activity as compared to control plants. In durum wheat cultivars maximal activity of CAT was observed at the milk ripeness and in bread wheat cultivars at the end of flowering. APX activity also increased in drought-treated leaves: in tolerant wheat genotypes maximal activity occurred at the end of flowering, in sensitive ones at the end of ear formation. GR activity increased in the tolerant cultivars under drought stress at all stages of ontogenesis. SOD activity significantly decreased in sensitive cultivars and remained at the control level or increased in resistant ones. This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: from Natural to Artificial.

1. Introduction

To cope with highly variable environmental stresses plants have to set a plethora of adaptation mechanisms, from early responses to longer term metabolic and physiognomic alterations that can sustain acclimation and survival [1,2]. Drought is a world-spread problem seriously influencing crop production and quality, the loss of which is the total for other natural disasters, with increasing global climate change making the situation more serious [3]. A wide range of strategies, which have been used to enhance the tolerance to drought depend on the genetically determined plant capacity and sensitivity, as well as on the intensity and duration of the stress [4]. Understanding the physiological and biochemical mechanisms providing drought tolerance is very important in terms of developing selection and breeding strategies.

Among crop plants, wheat is the staple food for more than 35% of world population and is often grown in water-limited conditions. Wheat anti-drought study is of importance to worldwide wheat production and biological breeding.

Drought stress leads to increased accumulation of reactive oxygen species (ROS) in plants. Various subcellular organelles such as chloroplast, mitochondrion and peroxisome are the common sites of ROS production. Increased levels of ROS cause damage to various cellular mechanisms, such as enzyme inhibition, protein degradation, DNA and RNA damage, and membrane lipid per-oxidation, which ultimately culminate in cell death [5].

Oxidative stress can lead to inhibition of the photosynthesis and respiration processes and, thus, plant growth. As the key process of primary metabolism, photosynthesis plays a central role in plant performance under drought, via decreased CO_2 diffusion to the chloroplast and metabolic constraints [1,6]. The ability to maintain the functionality of the photosynthetic machinery under water stress, therefore, is of major importance in drought tolerance. The plant reacts to water deficit with a rapid closure of stomata to avoid further loss of water through transpiration [7]. Several in vivo studies demonstrated that water deficit resulted in damages to the oxygen evolving complex of PS II [8] and

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; POD, peroxidase; F, fluorescence; GR, glutathione reductase; PS I, photosystem I; PS II, photosystem II; ROS, reactive oxygen species; RWC, relative water content; SOD, superoxide dismutase; Chl, chlorophyll; F_0, minimum fluorescence yield in the dark adapted state; F_m, maximum fluorescence yield in the dark adapted state

☆ This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: from Natural to Artificial.

* Tel.: +994 12 538 1164; fax: +994 12 510 2433.
E-mail address: huseynova-i@botany-az.org.

0005-2728/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2012.02.037

to the PS II reaction centers associated with the degradation of D1 protein [9,10]. The balance between light capture and energy use are of great relevance to studies concerning the responsiveness of the photosynthetic apparatus under water-stress conditions [1,11]. When photosynthesis decreases and light excitation energy is in excess, photooxidative damage may occur. The excessive excitation energy in PSII will lead to an impairment of photosynthetic function, progress to an accumulation of ROS, and thereby result in oxidative stress [12,13]. Generally, water stress may damage oxygen-evolving complex of PS II and PS I reaction centers [14]. Chlorophyll fluorescence measurements have become a widely used method to study the functioning of the photosynthetic apparatus and a powerful tool to study the plant's response to environmental stress [15–17].

Modulation in the activities of antioxidant enzymes may be one of the important factors in tolerance of various plants to environmental stress. Many attempts have been made over the last two decades to protect photosynthesis against stress-induced inhibition by manipulation of component antioxidant enzymes, and an extensive literature exists showing that enhancing the capacity of the water–water cycle through genetic engineering, including the overexpression of SOD and GR, can improve the tolerance of plants to abiotic stress [18]. Overall, the enhancement of chloroplast antioxidant defenses has proved to be one of the most effective ways of protecting plant cells from abiotic stress [19]. When molecular O_2 undergoes reduction, it gives rise of ROS such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (•OH). Singlet oxygen ($^1O_2$), which may arise due to reaction of O_2 with excited chlorophyll molecules, is also considered as one of the potential ROS.

Plants have evolved a highly efficient antioxidative defense system, including different types of enzymatic and non-enzymatic systems to scavenge/detoxify reactive oxygen species [20]. Enzymatic antioxidants include superoxide dismutase, catalase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase and glutathione reductase. The commonly known non-enzymatic antioxidants are glutathione, ascorbate (both water soluble), carotenoids and tocopherols (low molecular weight lipid soluble) [21–24]. Genes encoding different types of antioxidants have been engineered in different plants for achieving enhanced drought tolerance [25]. Undoubtedly, engineering of genes coding for antioxidative enzymes has provided new insights into the role of these enzymes in plant cells in counteracting stress-induced ROS. Although ROS in plants are produced under normal growth conditions and their concentration remains low [26]. Thus, ROS are considered as cellular indicators of stresses as well as secondary messengers actively involved in the stress-response signaling pathways. The enhanced production of ROS in chloroplasts and peroxisomes is correlated with drastic changes in nuclear gene expression [27] that reveals the transfer of $^1O_2$-derived signals from the plastid to the nucleus [28]. 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 [29].

In view of a number of reports in the literature it is now evident that alteration in ROS scavenging systems may cause considerable modification in oxidative stress tolerance and, hence, changes in tolerance to abiotic stresses [30]. There are some reports on photochemical efficiency of PS II [31,32] and antioxidant mechanisms under drought stress in tolerant and susceptible cvs of crop species [33]. However, antioxidant defense mechanism together with the efficiency of PS II, especially at all stages of ontogenesis was not studied in wheat cultivars with different tolerance levels under drought.

The aim of this study was to investigate the effects of drought stress on pigment composition, photosynthetic efficiency of PS II and changes in enzyme activities of APX, CAT, GR, and SOD of leaves from wheat (*Triticum* L.) cultivars during ontogenesis.

2. Materials and methods

2.1. Plant material

Experiments were undertaken on the winter wheat cultivars differing in drought resistance. Two durum wheat (*Triticum durum* L.) cultivars, cv Barakatli-95, which is tolerant to drought, and cv Garagylchyg-2, which is drought sensitive; and two bread wheat (*Triticum aestivum* L.) cultivars, less sensitive to drought cv Azamatli-95, and drought sensitive cv Giymatli-2/17 were grown in the field over a wide area under normal water supply conditions (control) or subjected to drought by withholding irrigation. The plants were provided by Experimental Station of the Research Institute of Crop Husbandry (Baku, Azerbaijan). Different sensitivities of these cultivars to drought have been determined during some years in different regions of Azerbaijan based on grain yield [34–36].

2.2. Relative water content

Leaf relative water content (RWC) was estimated gravimetrically according to the method of Tambussi et al. [37].

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 Huseynova et al. [38]. The homogenate was filtered twice through four layers of cheesecloth. The filtrate was centrifuged at 200×*g* for 5 min and then the supernatant was 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 determination

The chlorophyll concentration was determined in 80% acetone extract [39]. Samples were frozen in liquid nitrogen and stored at −80 °C until required.

2.5. Assay of fluorescence parameters of PS II

Chlorophyll fluorescence parameters were measured at room temperature using laboratory-built set-up as described earlier [40]. Maximal variable fluorescence ($F_v = F_m - F_o$) and the photochemical efficiency of PS II (F_v/F_m) for dark adapted state were calculated.

2.6. Enzyme extracts and determination of enzyme activity

The seedlings were excised and rapidly weighed (1 g fr wt). For all enzyme extracts leaf material (1 g fr wt) was ground with a pestle in an ice-cold mortar using different specific enzyme buffers. The homogenates were filtered through four layers of cheesecloth, and then centrifuged at 4 °C for 20 min at 15000×*g*. The supernatant was collected and used for enzyme assays.

2.6.1. CAT

Catalase (EC 1.11.1.6) activity was determined by the decomposition of H_2O_2 ($\varepsilon = 39.4\ mM^{-1}\ cm^{-1}$) and measured spectrophotometrically by following the decrease in absorbance at 240 nm for 1 min as described by Kumar and Knowles [41]. The reaction mixture contained 50 mM phosphate buffer (pH 7.0) and 0.1 mM H_2O_2, and reaction was initiated by adding 25 µl enzyme extract.

2.6.2. APX

Ascorbate peroxidase (EC 1.11.1.11) activity was assayed by the method of Nakano and Asada [42]. The activity was measured as a decrease in absorbance at 290 nm for 30 s. The assay mixture consisted of 0.05 mM ASA, 0.1 mM H_2O_2, 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.6), and 0.3 mL enzyme extract.

2.6.3. GR

Glutathione reductase (EC 1.6.4.2) activity was determined by following the decrease in absorbance at 340 nm due to the glutathione-dependence of NADPH for 10 min [43]. The reaction mixture contained 1 mM EDTA, 0.2 mM NADPH and 0.5 mM GSSG, 100 mM phosphate buffer (pH 7.8), and 0.2 mL enzyme extract.

2.6.4. SOD

Superoxide dismutase (EC 1.15.1.1) 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 enzyme required to cause 50% inhibition of the rate of NBT reduction.

2.7. Protein content determination

The protein contents of the extracts were measured based on the method of Sedmak [44] with bovine serum albumin as a standard.

3. Results and discussion

Prolonged periods of drought caused significant losses in relative water content, leaf Chl content, photosynthetic efficiency and activities of antioxidant enzymes.

RWC declined in the leaves during drought stress starting from the flowering stage. The decrease in leaf RWC was found to be lowest in cvs Barakatli-95 and Azamatli-95, and highest in cvs Garagylchyg-2 and Giymatli-2/17. A greater decrease in RWC was observed at the wax ripeness stage of ontogenesis. It constituted 69% in tolerant durum wheat cv Barakatli-95; and 61% in non-tolerant cv Garagylchyg-2. Also RWC was 68% in tolerant bread wheat cv Azamatli-95 and 57% in non-tolerant cv Giymatli-2/17 (Fig. 1).

Pigment content was changing in all studied plants depending on the stage of ontogenesis. In durum wheat, both tolerant cv Barakatli-95 and sensitive cv Garagylchyg-2, chlorophyll content reached a maximum in control plants at the beginning of earing stage. In the bread wheat maximum was observed at the end of earing, which indicates that plants show the highest photosynthetic activity exactly at this stage of growth. The minimum chlorophyll content in all wheat cultivars was observed at the end of ontogenesis. Water shortage significantly impacted the pigment content in non-irrigated plants. The pigment content significantly decreased in all cultivars with increasing drought. The maximum pigment content in all non-irrigated plants was observed at the end of earing stage. The amount of chlorophyll in the non-irrigated tolerant cv Barakatli-95 was less than the same in control at all stages of ontogenesis (Fig. 2). And in non-irrigated sensitive cv Garagylchyg-2 it exceeded the same of the control at the stage of stalk emergency and end of earing. But in the meantime, the chlorophyll content decreased more significantly in cv Garagylchyg-2 than in cv Barakatli-95. The 1.6-fold reduction in the chlorophyll content was observed in cv Barakatli-95 and 2.8-fold reduction in Garagylchyg-2 at wax ripeness stage. Bread wheat cultivars also followed such a tendency: the chlorophyll content was reduced by 1.5-fold in the tolerant cv Azamatli-95 and by 3.2-fold in drought-sensitive cv Giymatli-2/17 under water shortage at the wax ripeness stage (Fig. 2). It also was less in stressed cv Azamatli-95 than in the control at all stages of ontogenesis, excepting the stalk emergency stage. cv Giymatli-2/17 maintained a relatively stable concentration of chlorophyll at the initial stages of development: the chlorophyll content was slightly higher than the control at the stalk emergence and beginning of earing, and it almost reached the control at the end of earing. Noticeable changes occurred only at the beginning of flowering. The lowest chlorophyll content was observed in cv Garagylchyg-2. The decreased level of chlorophyll content is caused by photoinhibition and photodestruction of pigments and pigment–protein complexes and destabilization of photosynthetic membrane both induced by drought.

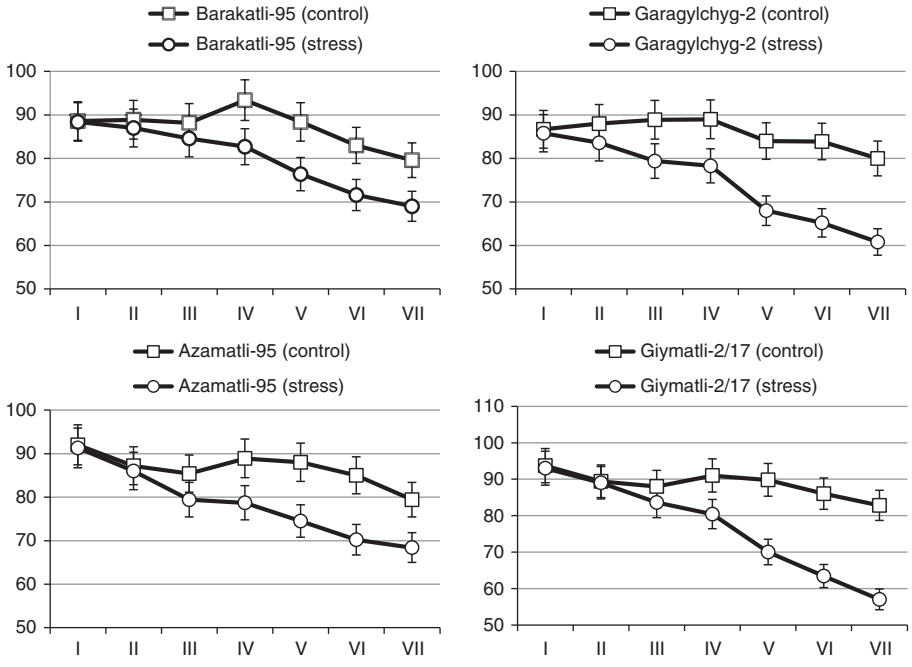

Fig. 1. Relative water content (%) of leaves in normally irrigated and drought stressed wheat plants at different stages of ontogenesis (I — stalk emergence, II — beginning of earing, III — end of earing, IV — beginning of flowering, V — end of flowering, VI — milky ripeness, VII — wax ripeness).

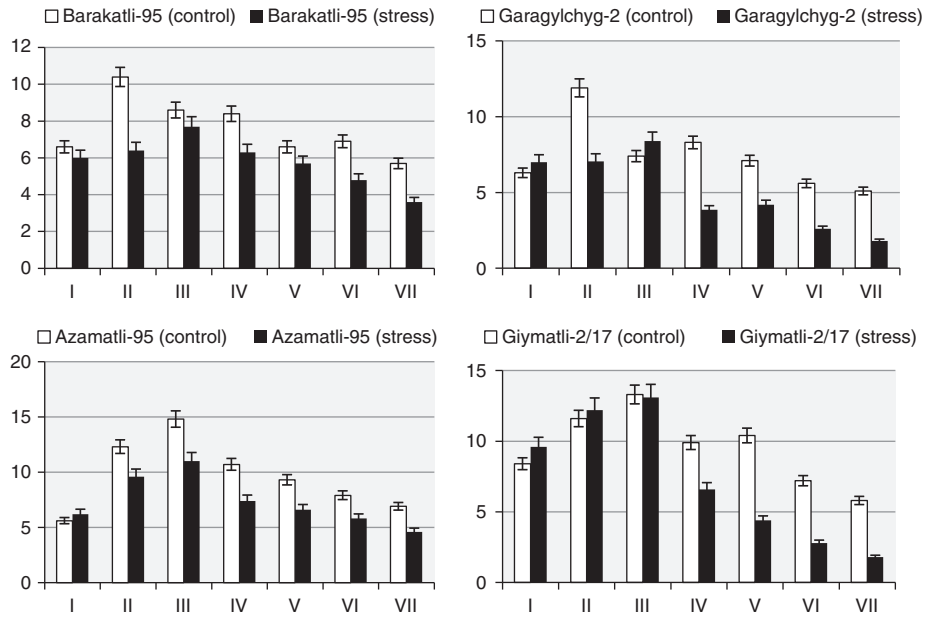

Fig. 2. Chlorophyll content (mg g^{-1} DW) of leaves in normally irrigated and drought stressed wheat plants at different stages of ontogenesis (I — stalk emergence, II — beginning of earing, III — end of earing, IV — beginning of flowering, V — end of flowering, VI — milky ripeness, VII — wax ripeness).

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 (RC) 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 irrigation are shown in Table 1. At the tillering stage when the drought variant still was absent, high photochemical activity was observed in cv Garagylchyg-2 (0.730 ± 0.024). F_v/F_m ratio in cv Barakatli-95 at the stage of stalk emergency under water deficiency showed a slight tendency to increase in comparison with fully irrigated plants. The maximum potential quantum yield of PS II in cv Barakatli-95 was observed at the stage of earing during drought ($F_v/F_m = 0.820 \pm 0.018$). It was observed that this ratio declined starting the flowering stage. It decreased to a minimum at the grain filling stage in plants subjected to water stress (0.610 ± 0.030). Difference in F_v/F_m ratio in cv Garagylchyg-2 was observed at the first days of drought, it was equal to 0.790 ± 0.021 in control plants and 0.840 ± 0.021 in water-stressed ones at the stage of stalk emergency. At the milky ripeness stages, when drought is the more severe, the potential quantum yield of PS II in cv Garagylchyg-2 significantly decreased compared with control plants. The maximum quantum yield of PS II in cv Azamatli-95 was observed at the stage of stalk emergency: $F_v/F_m = 0.850 \pm 0.024$ in the control plants and 0.870 ± 0.018 in stressed ones. F_v/F_m ratio was less in stressed plants than in control ones from the stage of earing until the end of the wax ripeness. In sensitive cv Giymatli-2/17, unlike other genotypes, Fv/F_m ratio decreased relatively to control at all stages of ontogenesis under stressful conditions. In plants subjected to drought stress the highest value of F_v/F_m ratio was observed at the stage of earing (0.790 ± 0.024). On the contrary, the ratio increased in the control plants and decreased in the stressed ones at the stage of flowering.

So, the F_v/F_m ratio, which characterizes the maximal quantum yield of the primary photochemical reactions in dark adapted leaves, was insignificantly decreased in the most stages of ontogenesis in all genotypes. A slight decrease in this parameter is the result of a large proportion of absorbed light energy not being used by the plants in the photosynthesis process. Only by the end of the drought period, a strong decrease was observed in the F_v/F_m . The decrease in PS II photochemistry reactions was more pronounced in the sensitive cultivars than in the resistant ones. The decrease in the F_v/F_m implies a decrease in the capture and conversion rate of excitation energy by PS II reaction centers and so, a reduction in PS II photochemical efficiency. These results indicate the disorganization of PS II reaction centers under the water stress conditions. The disorder appeared to be highly pronounced in sensitive cultivars than in resistant ones. Hence, obtained data showed that the inhibition of photosynthesis in droughted plants grown in the field is caused not only by injure of both thylakoid membrane electron transport, but also by other factors.

Table 1
Photochemical efficiency of PSII (F_v/F_m) in chloroplasts from well-watered and drought stressed wheat plants at different stages of ontogenesis.

Genotypes	Tillering	Stalk emergence	Earing	Flowering	Milky ripeness	Wax ripeness	Grain filling
Barakatli-95 (control)	0.668 ± 0.027	0.755 ± 0.024	0.771 ± 0.024	0.826 ± 0.021	0.795 ± 0.021	0.770 ± 0.021	0.742 ± 0.021
Barakatli-95 (drought)	–	0.774 ± 0.030	0.820 ± 0.018	0.770 ± 0.024	0.750 ± 0.024	0.737 ± 0.024	0.610 ± 0.030
Garagylchyg-2 (control)	0.730 ± 0.024	0.790 ± 0.021	0.818 ± 0.018	0.832 ± 0.018	0.779 ± 0.021	0.773 ± 0.024	0.70 ± 0.027
Garagylchyg-2 (drought)	–	0.840 ± 0.021	0.836 ± 0.018	0.760 ± 0.024	0.732 ± 0.024	0.720 ± 0.024	*
Azamatli-95 (control)	0.680 ± 0.027	0.850 ± 0.024	0.799 ± 0.021	0.819 ± 0.018	0.760 ± 0.021	0.771 ± 0.021	0.720 ± 0.024
Azamatli-95 (drought)	–	0.870 ± 0.018	0.758 ± 0.024	0.752 ± 0.024	0.724 ± 0.024	0.720 ± 0.024	*
Giymatli-2/17 (control)	0.695 ± 0.024	0.804 ± 0.024	0.832 ± 0.021	0.839 ± 0.018	0.810 ± 0.018	0.790 ± 0.021	0.750 ± 0.024
Giymatli-2/17 (drought)	–	0.770 ± 0.024	0.790 ± 0.024	0.778 ± 0.021	0.760 ± 0.021	0.748 ± 0.024	*

P.S. [–] — drought absent, [*] — the leaves were dried up.

Both Q_B-reducing and Q_B-non-reducing complexes of PS II make a contribution in variable fluorescence (F_v). Charge separation is realized in Q_B-non-reducing complexes of PS II, but electrons are not transported to plastoquinone pool. Q_B-reducing complexes of PS II in active state are able to realize electron transport between Q_A and Q_B [45]. They lose this ability when D_1-protein is damaged and turn to Q_B-non-reducing complexes [46]. In optimal conditions due to reactions of reparation cycle the constant ratio between these types of complexes of PS II is supported [47]. Probably, dehydration induces disruption of reactions at the acceptor side of PS II, expressed in increasing of a number of Q_B-non-reducing centers.

As Baker and Horton [48] mentioned, two distinct phenomena at least, are involved in producing the changes in the fluorescence parameters under unfavorable environmental conditions. One phenomenon results in an increase in F_0, possibly due to the reduced plastoquinone acceptor (Q_a^-), being unable to be oxidized completely because of retardation of the electron flow through PS II [49], or to the separation of light-harvesting Chl a/b protein complexes of PS II from the PS II core complex [50]. The second is responsible for quenching both F_v and F_m. Preferential quenching of F_v would indicate more extensive damage to the reaction centers, such that charge recombination is prevented. The drop of F_m may be associated with processes related to a decrease in the activity of the water-splitting enzyme complex and perhaps a concomitant cyclic electron transport within or around PS II [51]. Gilmore and Björkman [52] have pointed out that increased non-radiative energy dissipation would be expected to be accompanied by a quenching of F_m.

Under the stress conditions production of ROS is greatly increased and the oxidative burst occurs. The leading role in protecting the plants from ROS belongs to antioxidant enzymes. The results presented in this chapter are the first of such kind of studies of the dynamics of changes in the antioxidant enzymes activity in durum and bread wheat cultivars during all stages of ontogenesis under normal water supply and water deficit.

The functional dynamics of catalase in well-watered plants through ontogenesis differed among both of durum and bread wheat cultivars. Catalase, mostly localized in peroxisomes, breaks down and detoxifies H_2O_2. Our data showed that CAT exhibited maximal activity at the milk ripeness in cvs Barakatli-95 and Garagylchyg-2, and at the end of flowering in cvs Azamatli-95 and Giymatli-2/17. Functioning of the enzyme during ontogenesis increased under water deficit. CAT activity increased more substantially in drought-tolerant genotypes as compared to sensitive ones. Highest values of CAT activity were obtained during ontogenesis in Barakatli-95 (Fig. 3). The increase of CAT activity in plants under water stress was also reported in other studies [53,54]. It is known that CAT reacts with H_2O_2 directly to form water and oxygen [55]. The decrease in CAT activity at the end of ontogenesis could indicate its inactivation by the accumulated hydrogen peroxide under water shortage and could be partly explained 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 [56] conceivably could impair resynthesis and partly account for the marked decrease in CAT activity in plants subjected to water stress in the light.

The H_2O_2 scavenging enzyme, APX, located in both cytosol and chloroplasts can remove H_2O_2 efficiently, especially in the chloroplast where CAT is absent. Functional dynamics of ascorbate peroxidase in irrigated plants during ontogenesis were similar to that of CAT (Fig. 4). APX activity was higher in cv Barakatli-95 under water deficit during ontogenesis in comparison to the control: maximal activity occurred at the end of flowering, demonstarting the highest value of APX activity among all studied genotypes under drought. APX activity under drought was lower than the control in non-tolerant cv Garagylchyg-2. In tolerant cv Azamatli-95 it increased in comparison to control only at the end of flowering. In non-tolerant cv Giymatli-2/17, conversely, enzyme activity was higher than the control during the all stages of ontogenesis, except early stages (Fig. 4). An increase of POD activity was also observed by different authors during a drought and salt stress [57,58]. It indicates the formation of large amounts of H_2O_2 during water stress. Increase of peroxidase activity can be explained with some assumptions: elevated H_2O_2 concentrations could release POD from membrane structures normally associated with it. POD could be synthesized de novo at least in some cases. Water stress as other stress factors could increase the accumulation of POD substrates. Also it is known, that H_2O_2 participates in signal transduction at development of oxidizing stress, inducing genes of cytosolic POD.

GR also may remove H_2O_2 within chloroplasts by maintaining more favorable levels of reduced and oxidized glutathione. According to the obtained results, the maximal activity of GR in all genotypes under normal water supply was observed at the stage of flowering

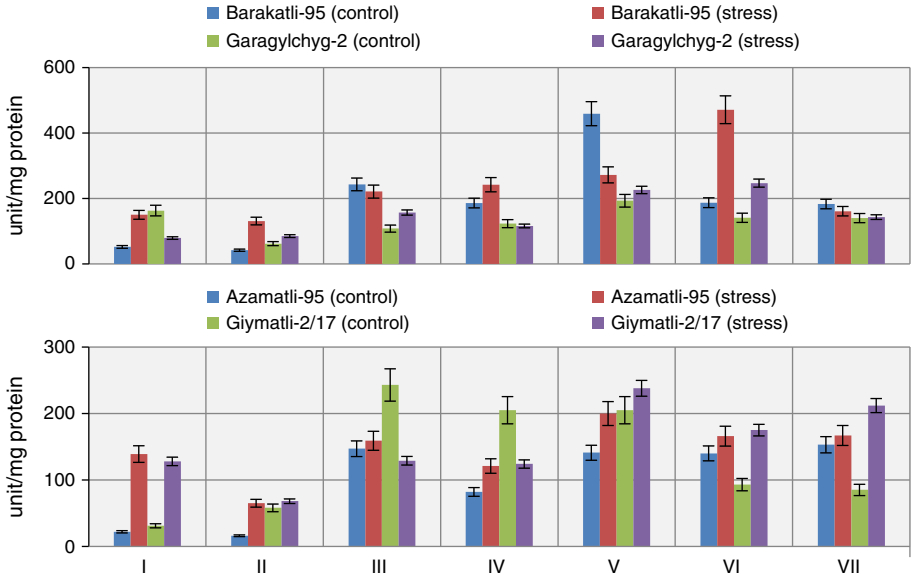

Fig. 3. Changes in CAT activity (enzyme unit (mg protein)$^{-1}$) of wheat plants in response to drought at different stage of ontogenesis (I — stalk emergence, II — beginning of earing, III — end of earing, IV — beginning of flowering, V — end of flowering, VI — milky ripeness, VII — wax ripeness).

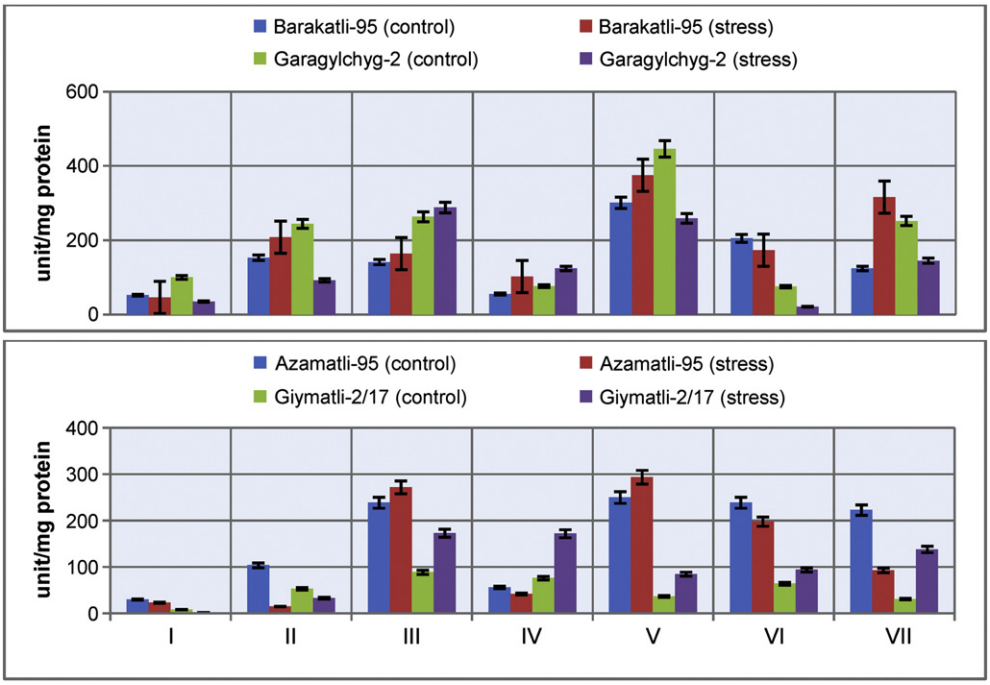

Fig. 4. Changes in APX activity (enzyme unit (mg protein)$^{-1}$) of wheat plants in response to drought at different stage of ontogenesis (I — stalk emergence, II — beginning of earing, III — end of earing, IV — beginning of flowering, V — end of flowering, VI — milky ripeness, VII — wax ripeness).

and was highest in cv Barakatli-95. The lowest GR activity was observed in cv Garagylchyg-2 at the wax ripeness. GR activity in tolerant varieties under the water deficit was higher than the control during ontogenesis (Fig. 5).

Maximal activity of superoxide dismutase in durum wheat genotypes under the normal water supply was observed at wax ripeness stage, while the minimal one — at the end of flowering (Fig. 6). Decrease of SOD activity to its minimum was also observed at the end of flowering in bread wheat cultivars. Maximal SOD activity was observed at the end of earing in cv Azamatli-9, and at the beginning of the earing in cv Giymatli-2/17. Drought strongly affected SOD activity. In tolerant cvs Barakatli-95 and Azamatli-95 SOD activity under the drought was the same as control (or slightly higher) at the end of ontogenesis, but in non-tolerant cvs Garagylchyg-2 and Giymatli-2/17 it decreased significantly in comparison to non-stressed plants (Fig. 6). SOD catalyzes the dismutation of superoxide anion radical ($O_2^{\bullet -}$) with great efficiency, resulting in the production of H_2O_2 and O_2. Many authors specify key role of SOD in antioxidative protection [59,60]. Because measured enzyme activity is a result of both synthesis and degradation, any decrease in net SOD activity under drought can be ascribed to either reduced synthesis or enhanced degradation of the enzyme. In addition, accumulation of H_2O_2 under drought also could lower SOD activity [61]. It is known that plant cells contain a little isoforms 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

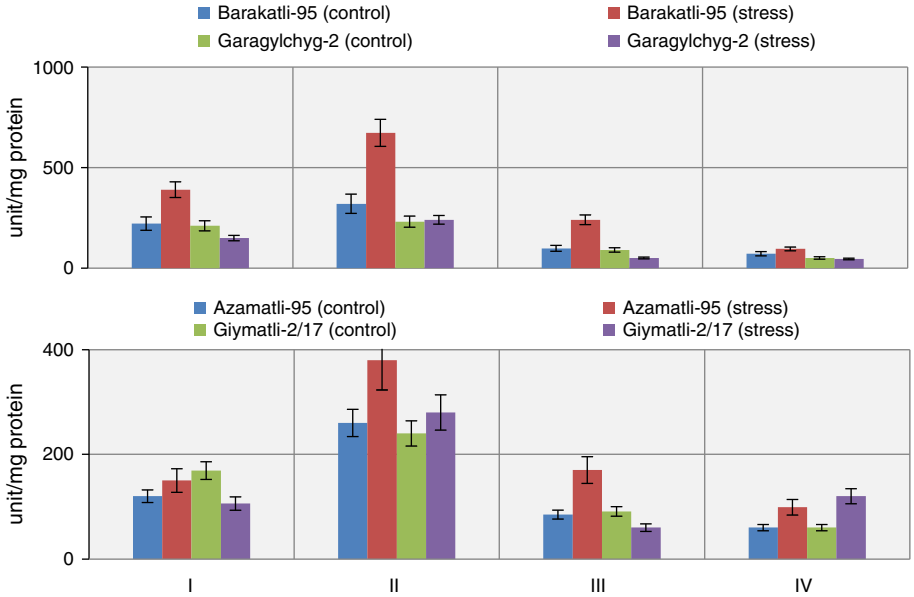

Fig. 5. Changes in GR activity (enzyme unit (mg protein)$^{-1}$) of wheat plants in response to drought at different stage of ontogenesis (I — stalk emergence, II — beginning of earing, III — end of earing, IV — beginning of flowering, V — end of flowering, VI — milky ripeness, VII — wax ripeness).

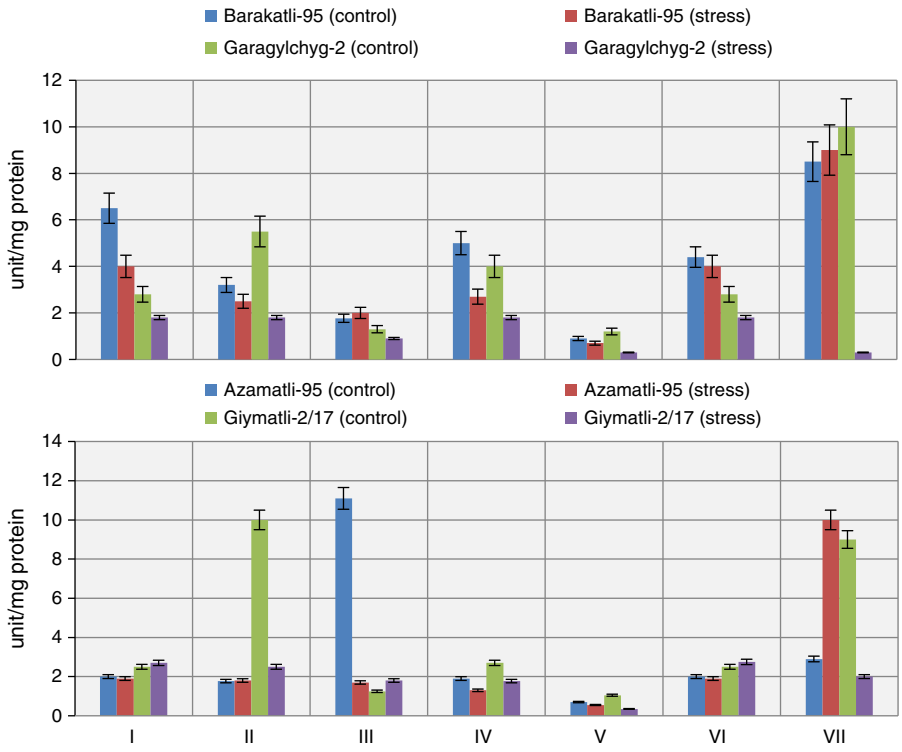

Fig. 6. Changes in SOD activity (enzyme unit (mg protein)$^{-1}$) of wheat plants in response to drought at different stage of ontogenesis (I — stalk emergence, II — beginning of earing, III — end of earing, IV — beginning of flowering, V — end of flowering, VI — milky ripeness, VII — wax ripeness).

FeSOD activities increased rapidly, while Cu/ZnSOD activities decreased in cowpea plants [62]. These results demonstrate a clear relationship between genetically determined tolerance of wheat genotypes and the level of antioxidant enzyme activities.

So, soil water deficit involves many changes in wheat (*Triticum* L.) genotypes resulting in decreased RWC, photosynthetic pigments content, activities of PS II and functioning of antioxidant enzymes CAT, APX, SOD and GR during ontogenesis. Photosynthesis is a well-established source of reactive oxygen species in plants. The photosynthetic electron transport chain operates in an aerobic environment; thus, regulatory systems are required to minimize ROS production. Moreover, an efficient antioxidant network is also essential in order to process ROS effectively and to maintain intracellular ROS pools at low levels. The obtained data showed that different wheat cultivars have had different photochemical efficiencies and used different antioxidant enzymes in order to scavenge reactive oxygen species. In tolerant cvs Barakatli-95 and 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 cvs Garagylchyg-2 and Giymatli-2/17. The more drought-sensitive cvs Garagylchyg-2 and Giymatli-2/17 responded to drought stress by reducing biomass accumulation, photosynthetic and antioxidant enzyme efficiency. Therefore, in cvs Barakatli-95 and Azamatli-95 the photosynthetic electron transport was probably sufficient to preclude the build-up of excess energy in PS II [31]. On the other hand, the more drought-tolerant cvs Barakatli-95 and Azamatli-95 seems able to avoid drought stress by maintaining a high photosynthetic activity and does not suffer an oxidative stress. Consequently, these cultivars also maintained a growth rate similar to those of well-watered control plants.

In our experiment obtained results showed that different wheat genotypes clearly responded to soil water deficiency differently in terms of activities of CAT, APX, SOD and GR.

It is evident that the increase in the level of antioxidative enzymes is at least one component of the mechanism of drought tolerance in most plants. The mechanism of drought tolerance in general, and mechanism of antioxidant production in particular, differ among species and even among cultivars of a single species. Furthermore, the form and functions of organs and tissue undergo a substantial time-course changes, so the capability of plants to respond to drought stress depends predominantly on the genes that are expressed at the stage of development during which the stress is imposed [63]. Dynamics of changes of antioxidative enzyme activity in wheat genotypes under normal water supply and deficiency of water at all stages of ontogenesis reveal that drought differently changes a balance between production of free radicals and enzyme reactions of protection. Obtained results allowed identification of correlation between genetically determined tolerance of wheat genotypes and the level of antioxidant enzymes activities.

The presented data might be used for monitoring environmental stresses in field grown plants and help in selecting stress-resistant varieties. Obtained results can suggest possible targets for the enhancement of stress tolerance in crops by genetic engineering.

Acknowledgement

This work was financially supported by grant from the Science Development Foundation under the President of the Republic of Azerbaijan (EIF-2010-1(1)-40/24-3).

References

[1] D.W. Lawlor, W. Tezara, Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes, Ann. Bot. 103 (2009) 561–579.
[2] I. Zaidi, C. Ebel, M. Touzri, K. Masmoudi, M. Hanin, TMKP1 is a novel wheat stress responsive MAP kinase phosphatase localized in the nucleus, Plant Mol. Biol. 73 (2010) 325–338.
[3] J.B. Passioura, The drought environment: physical, biological and agricultural perspectives, J. Exp. Bot. 58 (2007) 113–117.
[4] R. Mittler, E. Blumwald, Genetic engineering for modern agriculture: challenges and perspectives, Annu. Rev. Plant Biol. 61 (2010) 443–462.
[5] T. Ishikawa, K. Takahara, T. Hirabayashi, H. Matsumura, S. Fujisawa, R. Terauchi, H. Uchimiya, M. Kawai-Yamada, Metabolome analysis of response to oxidative

stress in rice suspension cells overexpressing cell death suppressor Bax inhibitor-1, Plant Cell Physiol. 51 (2010) 9–20.
[6] C. Pinheiro, M.M. Chaves, Photosynthesis and drought — can we make metabolic connections from available data? J. Exp. Bot. 62 (2011) 869–882.
[7] D.W. Lawlor, The effects of water deficit on photosynthesis, in: N. Smirnoff (Ed.), Environment and Plant Metabolism, Bios Scientific Publishers, Oxford, 1995, pp. 129–160.
[8] J. Scotnica, M. Matouskova, J. Naus, D. lazar, L. Dvorak, Thermoluminescence of fluorescence study of changes in photosystem II: a 100- to 200- ns component between 4.2 and 300 K, Proc. Natl. Acad. Sci. U. S. A. 77 (2000) 5889–5893.
[9] N. Murata, S. Takahashi, Y. Nishiyama, S. Allakhverdiev, Photoinhibition of photosystem II under environmental stress, Biochim. Biophys. Acta (BBA-Bioenergetics) 1767 (2007) 414–421.
[10] Z. Zlatev, Drought-induced changes in chlorophyll fluorescence of young wheat plants, Biotechnol. Biotechnol. Equip. (2009) 438–441 Special Edition.
[11] I. Aranjuelo, G. Molero, G. Erice, J.C. Avice, S. Nogués, Plant physiology and proteomics reveals the leaf response to drought in alfalfa (*Medicago sativa* L.), J. Exp. Bot. 62 (2011) 111–123.
[12] M.M. Chaves, J. Flexas, C. Pinheiro, Photosynthesis under drought and salt stress — regulation mechanisms from whole plant to cell, Ann. Bot. 103 (2009) 551–560.
[13] Y. Nishiyama, S.I. Allakhverdiev, N. Murata, Protein synthesis is the primary target of reactive oxygen species in the photoinhibition of photosystem II, Physiol. Plant. 142 (2011) 35–46.
[14] S.I. Allakhverdiev, N. Murata, Salt stress inhibits photosystems II and I in cyanobacteria, Photosynth. Res. 98 (2008) 529–539.
[15] A. Massacci, S.M. Nabiev, L. Pietrosanti, S.K. Nematov, T.N. Chernikova, K. Thor, J. Leipner, Response of the photosynthetic apparatus of cotton (*Gossypium hirsutum*) to the onset of drought stress under field conditions studied by gas-exchange analysis and chlorophyll fluorescence imaging, Plant Physiol. Biochem. 46 (2008) 189–195.
[16] S. Mathur, A. Jajoo, P. Mehta, S. Bharti, Analysis of elevated temperature induced inhibition of photosystem II using chlorophyll a fluorescence induction kinetics in wheat leaves (*Triticum aestivum*), Plant Biol. 13 (2011) 1–6.
[17] P. Mehta, A. Jajoo, S. Mathur, S. Bharti, Chlorophyll a fluorescence studies revealing effects of high salt stress on photosystem II in wheat leaves, Plant Physiol. Biochem. 48 (2010) 16–20.
[18] B.A. Logan, D. Kornyeyev, J. Hardison, S. Holaday, The role of antioxidant enzymes in photoprotection, Photosynth. Res. 88 (2006) 119–132.
[19] C.C. Chang, I. Slesak, L. Jorda, A. Sotnikov, M. Melzer, Z. Miszalski, P.M. Mullineaux, J.E. Parker, B. Karpinska, S. Karpinski, Arabidopsis chloroplastic glutathione peroxidases play a role in cross talk between photooxidative stress and immune responses, Plant Physiol. 150 (2009) 670–683.
[20] Y. Jiang, B. Huang, Drought and heat stress injury to two cool-season turfgrasses in relation to antioxidant metabolisms and lipid peroxidation, Crop Sci. 41 (2001) 436–442.
[21] K. Asada, The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons, Ann. Rev. Plant Physiol. Plant. Mol. Biol. 50 (1999) 601–639.
[22] G. Kiddle, G.M. Pastori, S. Bernard, C. Pignocchi, J. Antoniw, P.J. Verrier, C.H. Foyer, Effects of leaf ascorbate content on defense and photosynthesis gene expression in *Arabidopsis thaliana*, Antioxid. Redox Signal. 5 (2003) 3–32.
[23] L. Gomez, H. Vanacker, P. Buchner, G. Noctor, C.H. Foyer, The intercellular distribution of glutathione synthesis and its response to chilling in maize, Plant Physiol. 134 (2004) 1662–1671.
[24] K.J. Gupta, M. Stoimenova, W.M. Kaiser, In higher plants, only root mitochondria, but not leaf mitochondria reduce nitrite to NO, in vitro and in situ, J. Exp. Bot. 56 (2005) 2601–2609.
[25] M. Ashraf, Inducing drought tolerance in plants: recent advances, Biotechnol. Adv. 28 (2010) 169–183.
[26] A. Polle, Dissecting the superoxide dismutase–ascorbate–glutathione pathway in chloroplasts by metabolic modeling. Computer simulations as a step towards flux analysis, Plant Physiol. 126 (2001) 445–462.
[27] I. Gadjev, S. Vanderauwera, T.S. Gechev, C. Laloi, I.N. Minkov, V. Shulaev, K. Apel, D. Inze, R. Mittler, F. Van Breusegem, Transcriptomic footprints disclose specificity of reactive oxygen species signaling in Arabidopsis, Plant Physiol. 141 (2006) 436–445.
[28] A. Laloi, K. Apel, A. Dannon, Reactive oxygen signaling: the latest news, Curr. Opin. Plant Biol. 7 (2004) 323–328.
[29] A. Baruah, K. Simkova, K. Apel, C. Laloi, Arabidopsis mutants reveal multiple singlet oxygen signaling pathways involved in stress response and development, Plant Mol. Biol. 70 (2009) 547–563.
[30] G.M. Pastori, C.H. Foyer, Common components, networks and pathways of cross tolerance to stress. The central role of "redox" and abscisic acid-mediated controls, Plant Physiol. 129 (2002) 460–468.
[31] B. Loggini, A. Scartazza, E. Brugnoli, F. Navari-Izzo, Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought, Plant Physiol. 119 (1999) 1091–1100.
[32] H. Nar, A. Saglam, R. Terzi, Z. Varkonyi, A. Kadioglu, Leaf rolling and photosystem II efficiency in *Ctenanthe setosa* exposed to drought stress, Photosynthetica 47 (2009) 429–436.
[33] H.B. Shao, Z.S. Liang, M.A. Shao, Q. Sun, Dynamic changes of antioxidative enzymes of 10 wheat genotypes at soil water deficits, Colloids Surf. B Biointerfaces 42 (2005) 187–195.
[34] J.A. Aliev, Importance of photosynthesis of various organs in protein synthesis in grain of wheat genotypes under water stress, in: G. Garab (Ed.), Proceedings of the XIth International Congress on Photosynthesis, Budapest (Hungary), 1998, Photosynthesis: Mechanisms and Effects, vol. V, Kluwer Academic Publishers, Dordrecht, Boston, London, 1998, pp. 3829–3832.
[35] J.A. Aliev, Physiological bases of wheat breeding tolerant to water stress, in: Z. Bedo, L. Lang (Eds.), Proceedings of the 6th International Wheat Conference, Budapest, Hungary, 2000, Wheat in a Global Environment, vol. 9, Kluwer Academic Publishers, Dordrecht, Boston, London, 2001, pp. 693–698.
[36] J.A. Aliyev, Wheat breeding in Azerbaijan, Proceedings of Azerbaijan National Academy of Sciences (Biological Sciences), 3–4, 2006, pp. 3–32, (in Russian).
[37] E.A. Tambussi, S. Nogues, J.L. Araus, Ear of durum wheat under water stress: water relations and photosynthetic metabolism, Planta 221 (2005) 446–458.
[38] I.M. Huseynova, S.Y. Suleymanov, J.A. Aliyev, Structural–functional state of thylakoid membranes of wheat genotypes under water stress, Biochim. Biophys. Acta 1767 (2007) 869–875.
[39] G. Mc-Kinney, Absorption of light by chlorophyll solutions, J. Biol. Chem. 140 (1941) 315–322.
[40] V.V. Klimov, S.I. Allakhverdiev, V.A. Shuvalov, A.A. Krasnovsky, Effect of extraction and re-addition of manganese on light reactions of photosystem II preparations, FEBS Lett. 148 (1982) 307–312.
[41] C.N. Kumar, N. Knowles, Changes in lipid peroxidation and lipolytic and free-radical scavenging enzyme during aging and sprouting of potato (*Solanum tuberosum L.*) seed-tubers, Plant Physiol. 102 (1993) 115–124.
[42] Y. Nacano, K. Asada, Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts, Plant Cell Physiol. 22 (1981) 867–880.
[43] G.G. Yannarelli, A.J. Fernandez-Alvarez, Glutatione reductase activity and isoforms in leaves and roots of wheat plants subjected to cadmium stress, Phytochemical 68 (2007) 505–512.
[44] J.J. Sedmak, S.E. Grossberg, A rapid, sensitive, and versatile assay for protein using Coomassie brilliant blue G 250, Anal. Biochem. 79 (1977) 544–552.
[45] S.I. Allakhverdiev, Recent progress in the studies of structure and function of photosystem II, J. Photochem. Photobiol., B 104 (2011) 1–8.
[46] N.L. Pshibytko, L.N. Kalitukho, L.F. Kabashnikova, Effect of water deficit and high temperature on photosystem II in *Hordeum vulgare* leaves of various ages, Russ. J. Plant Physiol. 50 (2003) 51–58.
[47] S.I. Allakhverdiev, D.A. Los, P. Mohanty, Y. Nishiyama, N. Murata, Glycinebetaine alleviates the inhibitory effect of moderate heat stress on the repair of photosystem II during photoinhibition, Biochim. Biophys. Acta (BBA-Bioenergetics) 1767 (2007) 1363–1371.
[48] N.R. Baker, P. Horton, Chlorophyll fluorescence quenching during photoinhibition, in: D.J. Kyle, C.B. Osmond, C.J. Arntzen (Eds.), Photoinhibition, Elsevier Scientific Publisher, Amsterdam, 1987, pp. 85–94.
[49] V. Velikova, T. Tsonev, I. Yordanov, Light and CO_2 responses of photosynthesis and chlorophyll fluorescence characteristics in bean plants after simulated acid rain, Physiol. Plant. 107 (1999) 77–83.
[50] A. Cona, T. Kučera, J. Masojídek, B. Geiken, A.K. Mattoo, M.T. Giardi, Long-term drought stress symptom: structural and functional reorganization of photosystem II, in: M. Mathis (Ed.), Photosynthesis: from Light to Biosphere, vol. IV, Kluwer Academic Publisher, Dordrecht- London, 1995, pp. 521–524.
[51] E.-M. Aro, I. Virgin, B. Andersson, Photoinhibition of photosystem II. Inactivation, protein damage and turnover, Biochem. Biophys. Acta 1143 (1993) 113–134.
[52] A.M. Gilmore, O. Björkman, Temperature-sensitive coupling and incoupling of Atpase-mediated, nonradiative energy dissipation: similarities between chloroplasts and leaves, Planta 197 (1995) 646–654.
[53] S.P. Mukherjee, M.A. Choudhuri, Implications of water stress-induced changes in the leaves of endogenous ascorbic acid and hydrogen peroxide in vigna seedlings, Physiol. Plant. 58 (1983) 166–170.
[54] M.F. Quartacci, C. Pinzino, C.L.M. Sgherri, F. Navari-Izzo, Lipid composition and protein dynamics in thylakoids of two wheat cultivars differently sensitive to drought, Plant Physiol. 108 (1995) 191–197.
[55] N. Smirnoff, The role of active oxygen in the response of plants to water deficit and desiccation, New Phytol. 125 (1993) 27–58.
[56] M. Badiani, M.G. De Biasi, M. Colognola, F. Artemi, Catalase, peroxidase and superoxide dismutase activities in seedlings submitted to increasing water deficit, Agrochimica 34 (1990) 90–102.
[57] B.Z. Siegel, Plant peroxidases: an organism perspective, Plant Growth Regul. 12 (1993) 303–312.
[58] G.W. Winston, Stress Responses in Plants: Adaptation and Acclimation Mechanisms, in: R.G. Alscher, J.R. Cumming (Eds.), , 1990, pp. 57–86, New York.
[59] R.G. Alscher, N. Erturk, L.S. Heath, Role of superoxide dismutases (SODs) in controlling oxidative stress in plants, Exp. Bot. 53 (2002) 1331–1341.
[60] S.S. Raychaudhuri, The role of SOD in combating oxidative stress in higher plants, Bot. Rev. 66 (2000) 89–98.
[61] J. Zhang, M.V. Kirkham, Drought–stress-induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species, Plant Cell Physiol. 35 (1994) 785–791.
[62] Y.C. Brou, A. Zeze, O. Diouf, M. Eyletters, Water stress induces overexpression of superoxide dismutases that contribute to the protection of cowpea plants against oxidative stress, Afr. J. Biotechnol. 6 (2007) 1982–1986.
[63] M. Ashraf, Biotechnological approach of improving plant salt tolerance using antioxidants as markers, Biotechnol. Adv. 27 (2009) 84–93.