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

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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

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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.