Plant Physiology and Biochemistry 81 (2014) 54–60

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Subcellular localization and responses of superoxide dismutase isoforms in local wheat varieties subjected to continuous soil drought

Irada M. Huseynova*, Durna R. Aliyeva, Jalal A. Aliyev

Institute of Botany, Azerbaijan National Academy of Sciences, 40 Badamdar Highway, Baku AZ1073, Azerbaijan

ARTICLE INFO

Article history:
Received 6 December 2013
Accepted 28 January 2014
Available online 6 February 2014

Keywords:
Triticum durum Desf.
Antioxidant enzymes
Drought
Lipid peroxidation
Glycine betaine
Superoxide dismutase

ABSTRACT

Water is a key factor influencing the yield and quality of crops. One of the parameters of plant biological tolerance to constantly changing environmental conditions is the change of activities and numerous molecular forms of antioxidant enzymes. Two durum (*Triticum durum* Desf.) wheat varieties contrasting for drought tolerance, such as Barakatli-95 (drought tolerant) and Garagylchyg-2 (drought sensitive) were grown over a wide area in the field. Experiments were carried out to study the effect of soil drought on changes in activities and subcellular localization of superoxide dismutase isoforms. The levels of malondialdehyde, glycine betaine and total proteins were also analyzed. The level of the enzyme activity appeared to depend on the wheat varieties, duration of drought and stages of leaf development. Native polyacrylamide gel electrophoresis (PAGE) revealed the presence of 9 isoenzymes of superoxide dismutase in wheat leaves during drought. Mn-SOD was found in the mitochondrial fractions, Fe-SOD in the chloroplast fraction and Cu/Zn-SOD is localized in all subcellular fractions. Wheat leaves contain three different isoforms of SOD (Mn-, Fe-, Cu/Zn-SOD). Three isoforms of Mn-SOD, one isoform of Fe-SOD and five of Cu/Zn-SOD were observed in wheat leaves using 3 mM KCN and 5 mM H_2O_2 as selective inhibitors. The expression of Mn-SOD was preferentially enhanced by drought stress. It seems that Mn-SOD isoforms more than SOD ones play a major role in the scavenging of superoxide radicals. The observed data showed that status of antioxidant enzymes such as SOD could provide a meaningful tool for depicting drought tolerance of wheat genotype.

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1. Introduction

Water deficiency is one of the most important environmental factors leading to a decline in crop yield all over the world. Therefore increasing the drought-tolerance is the main challenge for agronomy. Tolerance does not only mean to survive under water deficiency but also maintain productivity. Over the past few decades many countries have made striking advances to improve drought tolerance of plants (Li et al., 2009). However, there is no doubt that the tolerance of plants to water stress may provide different mechanisms, that efficiency depends on climatic conditions (Collins et al., 2008; Tester and Langridge, 2010; Ashraf M, 2010). Wheat is one of the commonly cultivated plants in Azerbaijan. It attracts attention of researchers more than any other crop because of the global climate changes, as drought is the main stress factor limiting productivity (Aliyev, 2012; Huseynova et al., 2013). Effects of progressive soil drought on physiological and biological processes in wheat were studied poorly. Thereby the investigation of drought influence on physiological and biological processes during ontogenesis is of great interest. Selection of wheat varieties with enhanced tolerance to stress factors is a main link in creation of new high productive genotypes. In this respect, biotechnological approaches, including identification and modification of genes, coding protective proteins are perspective. Recent investigations revealed positive correlation between biomass accumulation ability and productivity of plants under normal as well as water-deficiency conditions (Lafitte et al., 2007). There have been a number of reviews devoted to physiological mechanisms of drought tolerance, which assert that under moderate drought yield can be related to plant ability to maintain more than inhibit its growth (Tester and Langridge, 2010; Lafitte et al., 2007; Fischer and Edmeades, 2010). According to the literature modern varieties can combine high productivity under favorable conditions, with drought tolerance (Lafitte et al., 2007; Burke et al., 2006).

Abbreviations: ROS, reactive oxygen species; SOD, superoxide dismutase; LPO, lipid peroxidation; MDA, malondialdehyde; GB, glycine betaine; PMSF, phenylmethyl-sulfonyle fluoride; PVP, polyvinylpyrrolidone; BSA, bovine serum albumin; DTT, dithiothreitol; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NBT, nitro blue tetrazolium; EDTA-Na, ethylenediaminetetraacetic acid disodium salt; PAGE, polyacrylamide gel electrophoresis.

* Corresponding author. Tel.: +994 12 538 1164; fax: +994 12 510 2433.
E-mail addresses: huseynova-i@botany-az.org, i_guseinova@mail.ru (I.M. Huseynova).

http://dx.doi.org/10.1016/j.plaphy.2014.01.018
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Nonspecific protective systems contribute significantly to plant tolerance to the complex action of stress factors. The antioxidant system is especially important among protective systems. Considering the fundamental role of reactive oxygen species in the organism response and in a variety of protective reaction mechanisms, recently issues of possible managing plant tolerance have been actively discussed. Some authors detected a direct relation between the level of the antioxidant system induction and degree of plant drought tolerance (Lascano et al., 2001; Carvalho, 2008). However, this relation was not manifested in all experiments. Chinese scientists showed that activities of superoxide dismutase (SOD), ascorbate peroxidase and glutathione reductase in a tolerant wheat variety – *Elite* increased under drought conditions (Lascano et al., 2001). In contrast, in non-tolerant variety – *Oasis* activities of antioxidant enzymes decreased or did not change.

Superoxide dismutase (SOD; EC 1.15.1.1), decreasing superoxide concentration plays a principal role in the elimination of reactive oxygen species (ROS). The enzyme is present in plant cells, where oxidation–reduction processes occur. SOD is represented by three isoforms, differing with metal ions in their active centers, Mn-SOD, Fe-SOD and Cu/Zn-SOD. Comparison of data on localization of different SOD isoforms revealed, that Cu/Zn-SOD was the most abundant form in plant cells. It was detected in all intracellular compartments: in cytosol (Hurst et al., 2002), chloroplasts (Ogawa et al., 1996), mitochondria (Kuzniak and Sklodowska, 2004), peroxisomes (Corpas et al., 2001) and apoplasts (Ogawa et al., 1996). Mn-SOD is present in mitochondria (Kuzniak and Sklodowska, 2004) and peroxisomes (Palma et al., 1998), but Fe-SOD – in chloroplasts (Navari-Izzo et al., 1998) and in cytoplasm of nodules of some legumes (Moran et al., 2003). However, the exact subcellular localization of different SOD isoforms is still insufficiently investigated. Considering that SOD performs as the first line of the enzymatic protection system and its total activity is stipulated by contributions of different isoform types, the study of individual changes of its activity under drought conditions is extremely important.

The purpose of the present paper was to investigate the role of the antioxidant system in the constitutive and induced tolerance of wheat under long-term drought conditions. The activity and isoenzyme composition of the key enzyme of antiradical protection, superoxide dismutase as well as contents of MDA and GB were studied under physiologically normal and drought conditions. In our opinion, investigations of the cellular compartmentation of superoxide dismutase are of great importance for more profound understanding of physiological and biochemical mechanisms underlying plant resistance under stress conditions.

2. Materials and methods

2.1. Plant material

In the present work, two durum wheat (*Triticum durum* Desf.) genotypes contrasting in drought resistance – Barakatli-95 (drought-tolerant) and Garagylchyg-2 (drought-sensitive) taken from Gene Pool of Research Institute of Crop Husbandry (Baku, Azerbaijan) were used. Plants were grown in the fields under normal and drought conditions. Drought was induced by ceasing watering. Assays were conducted in all phases of onto-genesis.

2.2. Determination of the dry matter content

The method is based on the drying the weighed sample to a constant weight at 103 ± 2°C. Content of the dried matters in the examined sample was expressed as a percentage of the initial sample mass. The dry matter content was calculated using the formula:

$$C(\%) = \left(m_{dry}/m_{fresh}\right)\cdot 100$$

2.3. Isolation of subcellular fractions

All procedures were performed at 0–4°C. Isolation of the subcellular fractions was conducted using the method of differential centrifugation. The chloroplast isolation medium consisted of 0.35 M saccharose, 5 mM sodium ascorbate, 50 mM Tris-HCl buffer (pH 7.8), 1 mM $MgCl_2$, 5 mM DTT and 3 mM cysteine. The medium used to isolate mitochondria contained 0.35 M sucrose, 0.02 M HEPES buffer (pH 8.0), 5 mM EDTA-Na, 5 mM sodium ascorbate, 3 mM cysteine, 5% BSA, 1 mM $MgCl_2$. Cytoplasmic fraction was obtained after precipitation of mitochondria.

2.4. Enzyme extracts

The enzyme extract for SOD was prepared first by freezing 1 g of fresh leaves in liquid nitrogen to prevent proteolytic activity followed by grinding with 5 ml of extraction buffer (0.1 M phosphate buffer, pH 7.8, containing 1 mM EDTA, 2 mM PMSF, 1% PVP, 0.1% Triton Ч-100) and then filtrated through four layers of cheesecloth and centrifuged for 20 min at 15,000 g. The supernatant was collected and used for enzyme assays.

2.5. Determination of SOD activity

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 enzyme required to cause 50% inhibition of the rate of NBT reduction.

2.6. Determination of isoenzyme composition of SOD

The determination of isoform composition of SOD was performed by electrophoresis in 10% polyacrylamide gel under non-denaturating conditions in Tris–HCl buffer (pH 8.3), 4^0C, 3 h supply of constant current of 30 mA. Visualization of SOD isoforms was conducted according to Parida et al. (2004). The gel was incubated in 100 ml of 1.0 M Tris-HCl buffer (pH 8.2), containing 10 mg NBT, 75 mg EDTA-Na and 3 mg riboflavin, for 30 min in the dark. Then the gel was kept in the light until bright stripes appeared on the violet background. For the selective inhibition of SOD isoforms before the visualization the gel was incubated for 20 min in 3 mM KCN (inhibition of Cu/Zn-SOD) and 5 mM H_2O_2 (inhibition of Fe-SOD and Cu/Zn-SOD) (Miszalski et al., 1998). The gel was washed with distilled water to remove excess stain and was photographed.

2.7. Determination of glycine betaine content

Glycine betaine content was determined using the method of Greive and Grattan (1983). Optical density of the stained solution was measured with spectrophotometer at 365 nm. Betaine amount was defined from calibration curve, using commercial preparation (Serva) as a standard.

2.8. Determination of lipid peroxidation degree

Intensity of lipid peroxidation was evaluated according to the content of the compound interacting with 2-thiobarbituric acid (basically MDA), using for the analysis homogenate prepared in

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