Changes of Some Antioxidant Enzyme Activities in Leaves of Drought Tolerant Varieties of Wheat from Oltenia During Vegetation Stages

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Abstract: In Oltenia drought is becoming a major environmental constraint on crop production and one of the solicited characteristics in the present in these area is drought tolerance. With that end in view, this study aims to thoroughly experiment of investigated varieties in comparative crops in the specific pedological, climatic and technological conditions of Oltenia in order to point out the most stable and high performance genotypes. One of the earliest responses of plants to drought is the accumulation of active oxygen species such as superoxide, hydroxyl radicals, hydrogen peroxide and singlet oxygenes. Plants protect cell systems from the cytotoxic effects of these active radicals using enzymes such as superoxide dismutase, ascorbat peroxidase, glutathione reductase, catalase and non-enzymatic antioxidants: glutathione, ascorbic acid and carotenoids. The current paper presents the changes of anti-oxidative indices in leaves of five varieties of wheat during development time. We observed significant differences between behaviors from different genotypes.

INTRODUCTION

Drought is a multi-dimensional stress, which causes various physiological and biochemical effects on plants (Dhanda et al, 2000, Verhagen et al. 2004, Wang et al 2003). In Oltenia drought is becoming a major environmental constraint on crop production as annual rainfall is decreasing and for this it is important for breeders to consider crop drought resistance to ensure that their new varieties of wheat have the best opportunity for achieving their genetic potential. For this purpose, numerous researches are carried out to obtain and select new varieties adapted to certain crop conditions, increasing the productivity, ameliorating certain characters and increasing the resistance against diseases, pathogens or stress (Patnaik 2002). With that end in view, this study aims to thoroughly experiment of investigated varieties in comparative crops in the specific pedological, climatic and technological conditions of Oltenia (area characterized by a warm and drought climate) in order to point out the most stable and high performance genotypes. One of the earliest responses of plants to drought is the accumulation of active oxygen species such as superoxide, hydroxyl radicals, hydrogen peroxide and singlet oxygen (Apel et al 2004, Mittler 2002, Shigeoka et al, 2002). Plants protect cell systems from the cytotoxic effects of these active radicals using enzymes such as superoxide dismutase, ascorbat peroxidase, glutathione reductase, catalase and non-enzymatic antioxidants: glutathione, ascorbic acid and carotenoids (Foyer et al 1997, 2000). Peroxidase and catalase, enzymes ubiquitously spread in the living world, are heme-proteins implied in hydrogen peroxide scavenging in different ways. Catalase doesn’t require an additional substrate or a supply of reducing equivalents for its function as
peroxidase does. In the mean time, the different affinities of peroxidases (µM range) and catalase (mM range) for hydrogen peroxide suggest that they belong to two different classes of H$_2$O$_2$–scavenging enzymes: peroxidase might be responsible for the fine modulation of reactive oxygen intermediates (ROIs) for signaling, whereas catalase might be responsible for the removal of excess ROIs during stress (Moran et al. 1994, Shao 2005). Due to their abundance in plant tissues, peroxidase is one of the most studied plant enzymes. The physiological functions of peroxidase, such as lignifying enzyme (Ros Barcelo 1992), predominant stress enzyme (Du Pont et al. 2003), indolylacetic oxidase and many others are well described and understood.

This study is referring to the variation of catalase activity and peroxidase system activity in leaves of five varieties of wheat, during development time, in correlation with the proteins contents.

MATERIAL AND METHOD

Experiments were carried out on five varieties of Triticum aestivum (1: Dropia; 2: Boema; 3: Flamura 85; 4: Exotic; 5: S 9917-6) at Research and Agricultural Development Station from Simnic. These were chosen because they had a high productivity level in drought conditions. The „S.C.D.A.” geographical region Simnic is known as a drought area, with reduced rainfall and extremely high summer temperatures. These experiments were performed in highly-controlled technological condition. The plants have been sown at 16.10.2007, complexly fertilized 12-52-0 200 kg/ha (autumn) and treated with DIVIDENT 1l/t;

The soil from the investigated area is characterised by an acid pH (pH=5.7) by a low content of humus (1.8%), nitrogen (IN%=0.81), potassium (128 ppm) and by a significant supply of mobile phosphorus (54ppm). Biochemical analyses were realized on fresh leaves cut at different growing stages: faze I: leaf and tiller development, faze II: steam elongation, faze III: flag leaf.

Fresh tissue was homogenated with 0.1 M phosphate buffer (pH 7.0) containing 0.1 mM EDTA. Homogenates were centrifuged for 20 min at 10,000 r.p.m. and the supernatants were used for enzyme assay. The activity of ascorbate peroxidase was measures by determining the oxidative rate of ascorbic acid. Total soluble peroxidase (guaiacol-type E.C.1.11.1.7) was assayed by measuring the increase in A$_{436}$ due to the guaiacol oxidation and their activity was expressed as ∆A/min/1g fresh weight (Putter, 1974). Catalase (E.C.1.11.1.6) was assayed through the colorimetric method of Sinha (1972) and expressed as mmoles H$_2$O$_2$/min/g at 25°C. Protein concentration was evaluated by the method of Bradford using bovine albumin as a standard.

RESULTS AND DISCUSSION

The obtained results have showed a variation of biochemical indices analysed both on variety of wheat and on plant’s developmental stage. Even under optimal conditions, many metabolic processes occurring in plants produce active oxygen species which, besides their deleterious potential may act as signaling molecules. As catalase and peroxidase are parts of the antioxidant system of the cell, their activities can change drastically depending upon the physiological condition of the plant and the integration of different environmental, developmental and biochemical stimuli. Enzyme activities in the leaves of varieties of wheat are presented in Figures 1, 2 and 3. The data concerning enzyme activity variation sustains the idea that catalase activity remains high all of the time to degrade H$_2$O$_2$ at rapid rate. Decrease in catalase activity in the third phase leads to the increase of H$_2$O$_2$ level within the cell.
Total soluble peroxidase (guaiacol-type) and ascorbat peroxidase activity is dependent both on developmental stage and on studied variety of wheat. The guaiacol peroxidase activity (figure 2) increase in the second phase of vegetation and decrease in the third phase correlated with the soluble protein content (figure 4), suggesting an intensification of the biosynthesis process for each studied genotype in the second phase and activation of catabolic processes in the third vegetation phase.

The increase of ascorbat peroxidase activity from the third phase is negatively linked to the protein content’s variation and indicates an intensification of the catabolism. This observation is sustained by the catalase activity’s decrease from the third vegetative phase.

Among the studied varieties, the Exotic and S 9917-6 varieties that have a high content of proteins and a hight enzymatic activities are distinguished. The visual appreciation of the plots that have been experimented with these wheat varieties indicate a good overall aspect in the case of covering the plot with plants, of the foliar surface and a very good general aspect of kernels. The production biochemical indices are linked to the qualitative and quantitative ones, indicating a dependency between the intensification of the biosynthesis processes in the leaf and the protein accumulation process in the wheat kernels.
CONCLUSIONS

The analyzed biochemical indices show a dependency with the investigated phase and the studied genotype. The biochemical data presented confirm that the vegetation phase induces a modulation of the peroxidase system activity in plant leaves, that influences the peroxidase controlled processes.

Catalase activity was low influenced with the vegetation phase suggesting that it’s activity is less important during stages when peroxidase is active, this fact confirming the hypothesis of the alternative activation and inhibition of the two hem-enzymes.

The Exotic and S 9917-6 varieties with a high soluble protein content and high peroxidase activity are distinguished, indicating a good metabolical status, in pedological conditions that are specific to the investigated area, which recommends them for the selection of high productivity varieties.

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