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GENOTYPIC VARIATION IN ANTIOXIDANT ENZYME ACTIVITIES DURING DROUGHT AND RECOVERY PERIODS IN *TRITICUM AESTIVUM* L.

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Abstract

A comparative study was performed to understand the expression profile of antioxidant enzymes (*guaiacol peroxidase-GPX*, catalase-CAT and superoxide dismutase-SOD) during drought and r*ecovery* periods in drought-tolerant (HI1500) and drought-sensitive genotypes (LOK1) of *Triticum aestivum* L. Both the genotypes exhibited differential responses during drought and recovery period. During drought treatment, HI1500 exhibited significantly higher activity levels of *GPX, CAT* and *SOD* compared with *LOK1*. Moreover, Hi1500 recovered the enzymatic activity more rapidly than *LOK1*. The results clearly indicate that regulation of enzymatic activity under drought is an essential biochemical adaptive mechanism to prevent the plants from oxidative stress and status of antioxidant enzymes could provide a meaningful tool for depicting drought tolerance in wheat genotypes.

Key words : *Triticum aestivum*, Drought, Rehydration, Antioxidant enzymes.

Introduction

Drought is one of the most crucial abiotic stresses to plant growth and development. It leads to increase accumulation of reactive oxygen species (ROS) and causes inhibition to various molecular, biochemical and physiological processes in plants. Drought induces oxidative stress resulting from increased production of reactive oxygen species (ROS), including superoxide anion radical ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and hydroxyl radical ($^{\bullet}OH$). *Excessive* level of ROS can damage cellular lipids, proteins, or DNA, thus inhibiting signal transduction pathways and normal cellular functions (Cadet & Davies, 2017). To minimize and eliminate the ROS-mediated oxidative damage, plants have a sophisticated antioxidative defense system that includes the enzymes superoxide dismutase, ascorbate peroxidase, catalase, guaiacol peroxidase, peroxiredoxins, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase (Soares *et al*., 2019). However, changes in antioxidant enzyme activities under drought stress are dependent on plant species, cultivar, stress intensity and duration (Chmielewska *et al.,* 2016, Soni *et al.,* 2017).

Triticum aestivum L. is one of the main food crops, being consumed by humans for more than 5,000 years (Peng *et al.,* 2011). It provides nearly 55% of the carbohydrates requirements of the world population (Breiman & Graur, 1995). Drought adversely affects more than 50 percent of the wheat production area in the world (Parry *et al.,* 1999). Due to the importance of grain yields, less attention has been focused on studying the effects of drought during the vegetative growth periods of wheat. The degree of plant drought tolerance differs not only among various species but also among different varieties of the same species of wheat (Vassileva *et al.,* 2009). Therefore, in this research, our main objective was to determine the involvement of GPX, CAT and SOD during drought and r*ecovery* periods in drought-tolerant (HI-1500) and drought-sensitive (Lok-1) genotypes of *T. aestivum*. HI1500 is being cultivated in different parts of India under rainfed conditions, whereas LOK1 is sown under irrigated conditions. Understanding the biochemical basis of drought tolerance in HI1500 would help greatly

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in the development of appropriate wheat varieties for drought prone areas.

Material and Methods

Plant Materials and Growing Conditions

Drought-tolerant (HI1500) and drought-sensitive (Lok1) genotypes of *T. aestivum* were evaluated concerning their ability to endure drought stress. Seeds were obtained from Maharana Pratap Agriculture University and Technology, Udaipur (Rajasthan, India) and were sown *in vivo* in germination trays containing 50% clay, 25% sand, and 25% humus under controlled conditions at 15°C under a 12 h photoperiod. Prior to sowing, surface sterilization of seeds was done with 0.1% $HgCl_2$ followed successive washings with distilled water. Seedlings were watered twice a day.

Drought Treatment

The germinated plants were equally well watered for 2 weeks prior to exposure to drought stress treatment. After 3 weeks, at the stage of 2 fully developed leaves, the plants were divided into two sets (each of 50 plants), out of which one set was subjected to drought stress until the appearance of wilting symptoms by withholding of water supply, while the second set was watered regularly and served as a control.

Enzyme assay

Leaf tissues (0.5 g) were ground in 2 mL of 50 mmol L^1 potassium phosphate buffer (pH 7.0), 1 mmol L^1 ethylenediamine tetra acetic acid (EDTA), 0.1 mmol L^1 ascorbic acid (AA), 2% (w/v) polyvinylpyrrolidone (PVP), and 0.05% (w/v) Triton X-100 using a chilled pestle and mortar. The homogenate was centrifuged at 10,000 g for 20 min at 4^0C (Remi CM-12 Plus, India) and the supernatants were collected and used for the assays of *Guaiacol peroxidase* (GPX**,** *EC* 1.11.1.7) and catalase (CAT, EC 1.11.1.6). *POX* activity was measured spectrophotometrically by following the method of Racusen & Foote (1965). CAT was determined spectrophotometrically (Analytik Jena, Germany) by measuring the rate of H_2O_2 disappearance at 240 nm (Aebi, 1984). For assay of superoxide dismutase (SOD, *EC* 1.15.1.1), fresh leaves (1 g) were homogenized in 8 mL potassium phosphate buffer (50 mM, pH 7.8) containing 0.1 mM Na2–EDTA and 1% insoluble PVP with a chilled pestle and mortar. The homogenate was centrifuged at 20,000 g for 20 min. The supernatant was collected and used for the assay of SOD following the method of Beauchamp & Fridovich (1971) as modified by Giannopolitis & Ries (1977). One unit of SOD activity was defined as the amount of the enzyme that inhibited NBT reduction by 50% in comparison with tubes lacking enzymes.

Results and Discussion

A comparative biochemical study was carried out to analyze antioxidant enzymatic activities of drought-tolerant (HI1500) and drought-sensitive (Lok1) genotypes of *T. aestivum* subjected to a range of dehydration and rehydration treatment. In present study, the control plants displayed constant *GPX,* CAT and SOD activities throughout the experiment. Activities of all three antioxidant enzymes increased initially and then decreased in Lok1under drought treatment (Fig. 1A, B, C). On the other hand, the activities of *GPX,* CAT and SOD increased continuously in HI1500 with increasing drought period (Fig. 1D, E, F). Moreover, HI-1500 showed leaf rolling throughout the drought stress treatment.

Under drought stress, *GPX* activity increased significantly at 0–4 days in Lok1. After 4 days, a significant reduction in *enzyme* activity was observed until day 10 of drought stress (Fig. 1A). In contrast, mild drought stress could not modulate *GPX* activity in HI1500. The enzyme activity was increased continuously in HI-1500 during the exposure of drought stress. A sharp *increase* in *GPX* activity was observed at the 5^{th} *day* of drought stress (Fig. 1D). Plant recovery after rehydration is an essential trait for plant survival and reflects the balance between damaged structures reconstruction and adequate metabolism restoration (Moreira *et al.,* 1990). Therefore, comprehensive studies about plant responses to rehydration are essential. In present studies, Lok1 restored the *GPX* activity within 8 days of rehydration (Fig. 1A). On the other hand, a rapid recovery after rewatering was observed in HI1500 (Fig.1D). GPX activity was completely reversed to control level within 2 days of rehydration. CAT is a crucial enzyme to control the excess peroxisomal H_2O_2 produced during photorespiration. CAT activity significantly increased up to 4^{th} days of drought stress in Lok1. Later, its activity was drastically decreased reaching the lowest values on day 10^{th} of drought treatment (Fig. 1B). In contrast, CAT activity was enhanced up to 6 days of *drought treatment in HI-1500*. Thereafter, a slight reduction in enzyme activity was recorded up to the appearance of wilting symptoms (Fig.1E). The increase activity of catalase is an adaptive trait to overcome toxic levels of H_2O_2 and provide protection against oxidative stress (Chawla *et al.,* 2013). Upon rehydration, Lok1 slowly recovered CAT activity and reached control levels after 10 days of rewatering (Fig. 1B). In contrast, HI1500 showed a rapid restoration of CAT activity within 04 days of rehydration (Fig. 1D).

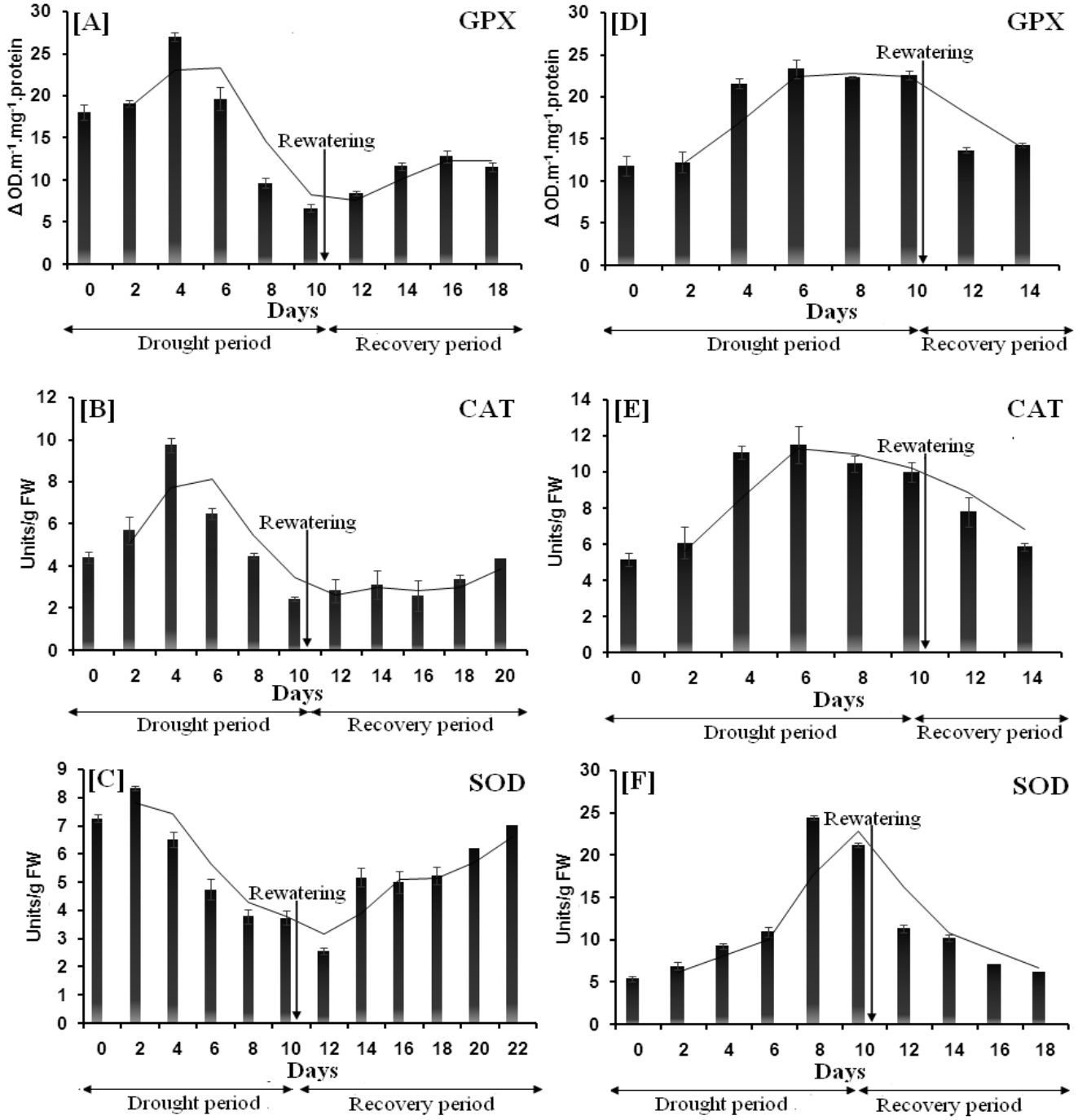

Fig.1: Expression of GPX, CAT and SOD during drought and r*ecovery* periods in drought-sensitive *LOK1 (A,C,E)* and drought-tolerant HI1500 *(B,D,F)* genotypes of *T. aestivum*.

SODs propel the disproportion of O^2 ion into hydrogen peroxide (H_2O_2) and oxygen (O_2) molecules. Thus, SODs play a critical role in protecting plant tissues from ROS (Alscher *et al.,* 2002). In present study, drought induced a slight increase in SOD activity in Lok1on 2nd day of drought treatment. Later on, its activity was declined continuously with increasing drought period (Fig. 1C). On the other hand, HI-1500 exhibited a significant increasing in SOD activity with drought duration. Highest SOD activity was observed on day 08 of drought stress in HI1500 (Fig. 1F). A wide variation in recovery period was observed in both tolerant and sensitive genotypes of *T. aestivum*. Lok1 and HI1500 recovered SOD activity to control level on 12th and 8th day of rewatering, respectively.

In present study, activities of *GPX,* CAT and SOD increased continuously in drought-tolerant genotype HI-1500 with increasing drought period until the appearance

of wilting symptoms. Similar enhanced activities of *GPX,* CAT and SOD was also observed in wheat (Khanna-Chopra. & Selote, 2007), *Capparis ovata* (Ozkur *et al.,* 2009) and peanut (Chakraborty *et al.,* 2016). Studies carried out by Zhang et al. (2004) in soybeans and wheat showed that after drought stress and rehydration, plants maintained high levels of CAT, SOD and APX activity to remove ROS effectively, consistent with the results in this study. Zhou & Deng (2007), Guo *et al*., (2008), and Zhang *et al.*, (2008) also found that rehydration after drought treatment significantly reduced the content of antioxidant substances such as proline, soluble sugars, and MDA that were accumulated during stress, consistent with the results in this study.

Conclusion

Present study reveals that expression level of antioxidant enzymes during drought and recovery periods determine drought tolerance to *T. aestivum* HI1500. Thus, status of antioxidant enzymes could be a very useful tool for depicting drought tolerance of wheat, which could be useful to plant breeders and biotechnologists for developing drought-tolerant genotypes. However, further studies are needed to confirm the role of antioxidant enzymes for depicting drought tolerance in a large number of genotypes.

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