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Antioxidant Isoenzymes Activities in Seedling Roots of Wheat Exposed to Drought Stress

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Abstract
This study was conducted to evaluate the role of oxidative stress in roots of eight wheat genotypes with different drought tolerance, subjected to drought stress. For analyzing the changes of antioxidant enzymes, native PAGE analyses of protein extract were performed. The roots of wheat seedlings showed one unambiguous isoform of superoxide dismutase (SOD) and catalase (CAT). Eight isoforms of peroxidase (POX) were identified in the roots of wheat. The activities of SOD and POX isoforms decreased and the total activities of POX and CAT remained unchanged under the stress condition. The response of enzyme isoforms to drought were not the same for all isoforms of the antioxidant enzymes in the wheat genotypes, as POX isoforms showed the significant changes in the different drought tolerant genotypes. Significant interaction was observed between wheat group and stress treatments for total POX activity. POX total activity in the sensitive group of wheat genotypes was significantly higher than the tolerant group in the stress condition.

Keywords: CAT; Drought stress; Isozyme; POX; SOD; Wheat

Introduction

Abiotic stresses such as drought, salinity and high temperature lead to oxidative stress in crop plants due to enhanced generation of reactive oxygen species (ROS) in different cell compartments (Mittler 2002). Plants have evolved antioxidant defense pathways to protect the cells from oxidative damage during the periods of normal growth as well as under stress conditions. Oxidative damage occurs either due to noninduction of coordinated antioxidant defense cascade or their mismanagement (Noctor and Foyer 1998). Membrane lipid peroxidation and/or protein oxidation are the simplest criteria of assessing the extent of oxidative stress in the tissue. Efficient antioxidant defense minimizes the level of oxidative stress in the cell (Selote and Khanna-Chopra 2006; Abogadallah and Serag 2010). The antioxidant molecules and enzymes, located in different cell compartments, scavenge ROS including superoxide dismutase (SOD; EC 1.15.1.1), which catalyzes the dismutation of $O_2^{\cdot-}$ to H_2O_2, and catalase (CAT; EC 1.11.1.6) and peroxidase (POX; EC 1.11.1.7), which are responsible for H_2O_2 removal. During drought stress, the plant water status plays a key role in the activation and/or modulation of antioxidant defense mechanism.

The measure of specific antioxidant enzyme activities and/or expression analysis during water stress treatments has been generally accepted as an approach to assess the involvement of the

scavenging system during drought stress. However, contradictory results have been observed through the years. These differences might be related to the plant age and tolerance/strategy towards water stress, but also to the duration and the intensity of the stress treatment. The plant roots are the first organs that sense the water-deficit condition (Davies and Zhang 1991) and thus become important tissues of plants to study the effect of drought. However, there are very few studies reported on root antioxidant enzymes accumulation and role in drought tolerance.

The present study was an effort to analyze antioxidant enzyme defense response in roots of eight genotypes of winter wheat with different drought tolerance.

Materials and Methods

Plant material and growth conditions: The plant material consisted of eight wheat genotypes with different response to drought (Table1). These genotypes were grown in a split plot design with four replications in the Research Station of the College of Agriculture, University of Tabriz, Iran during 2009–2010. Then, the harvested seeds were sown in petri dishes in the laboratory condition and seedling roots were analyzed for enzyme studies.

Table 1. Pedigree of eight wheat genotypes used in the study

Genotype code	Degree of tolerance	Pedigree
T1	Tolerant	Unknown-1
T2	Tolerant	Sabalan//84.40023/6149-27-1
T3	Tolerant	RECITL/TIA.//TRK13
T4	Tolerant	Vrz/3/Orfl.148/Tdl//Blo/4/Sabalan
S5	Sensitive	DARIC95-010-OMA-OMA-OMA-OMA-6MA-OMA
S6	Sensitive	HK16/7/KVZ/T171/3/MAYA//BB/INIA/4/KAR/JCWH99034-OAP-OAP-OAp-OMAR-6MAR
S7	Sensitive	FKG13/4/NWT/3/TAST/SPRW//TCI98-0139-OAP-OAP-OMAR-5MAR
S8	Sensitive	JANZ QT3685-OAUS

Enzyme extraction: For CAT and POX activity, roots bulked samples (500 mg fresh weight) were homogenized in pre-cooled mortars with 1 ml of extraction buffer (50 mM Tris, pH 7.5, 7% sucrose, 200 mM ascorbic acid, 20 mM sodium metabisulfite, 3% PEG6000 and freshly added 0.1% of 2-Mercaptoethanol). The homogenates were centrifuged at 12000 g for 15 minutes at 4°C and supernatants were used as enzyme sources.

Activity detection of enzymes isoforms by PAGE: Isoforms of CAT, POX and SOD were separated on non-denaturating (7%) polyacrylamide slab gels, using 76 mM Tris buffer (pH 8.8) containing 5.5 mM citric acid for gel formation and 32 mM Tris buffer (pH 8.8) containing 0.6 mM boric acid and 0.1 mM Na_2EDTA as electrode buffer. Nearly, 10 µl enzyme extracts were loaded by Whatman3 filter papers. Gel electrophoresis was performed at least

three times for each enzyme at 4°C for 3 h with a constant voltage of 150 V. POX isozymes were stained according to Anderson *et al.* (1995). For detection of SOD and CAT isoforms, the gels were stained according to Soltis and Soltis (1990). The gels were fixed and scanned immediately after staining. An image analysis program (MCID software) was used to measure the optical density (unitless, D) of each isozyme band. The band area (inch^2, A) was also determined by this program. Then D×A (optical density×area) parameter was calculated as densitometric isozyme activity (Ghassemi-Golezani *et al*. 2009).

Results

Antioxidant enzyme activities: Changes in the activity patterns of CAT, POX and SOD isozymes of winter wheat seedlings roots were visualized on native polyacrylamide slab gels (Figure 1). Two antioxidants, namely CAT and SOD, were observed as monomorphic isozymes (Figures 1A and 1B). The assessment of the POX isozyme profiles revealed the presence of eight isoforms in the winter wheat seedling roots for which mother plants were exposed to drought stress. These isoforms were subscripted by numbers 1 to 8 (Figure 1C).

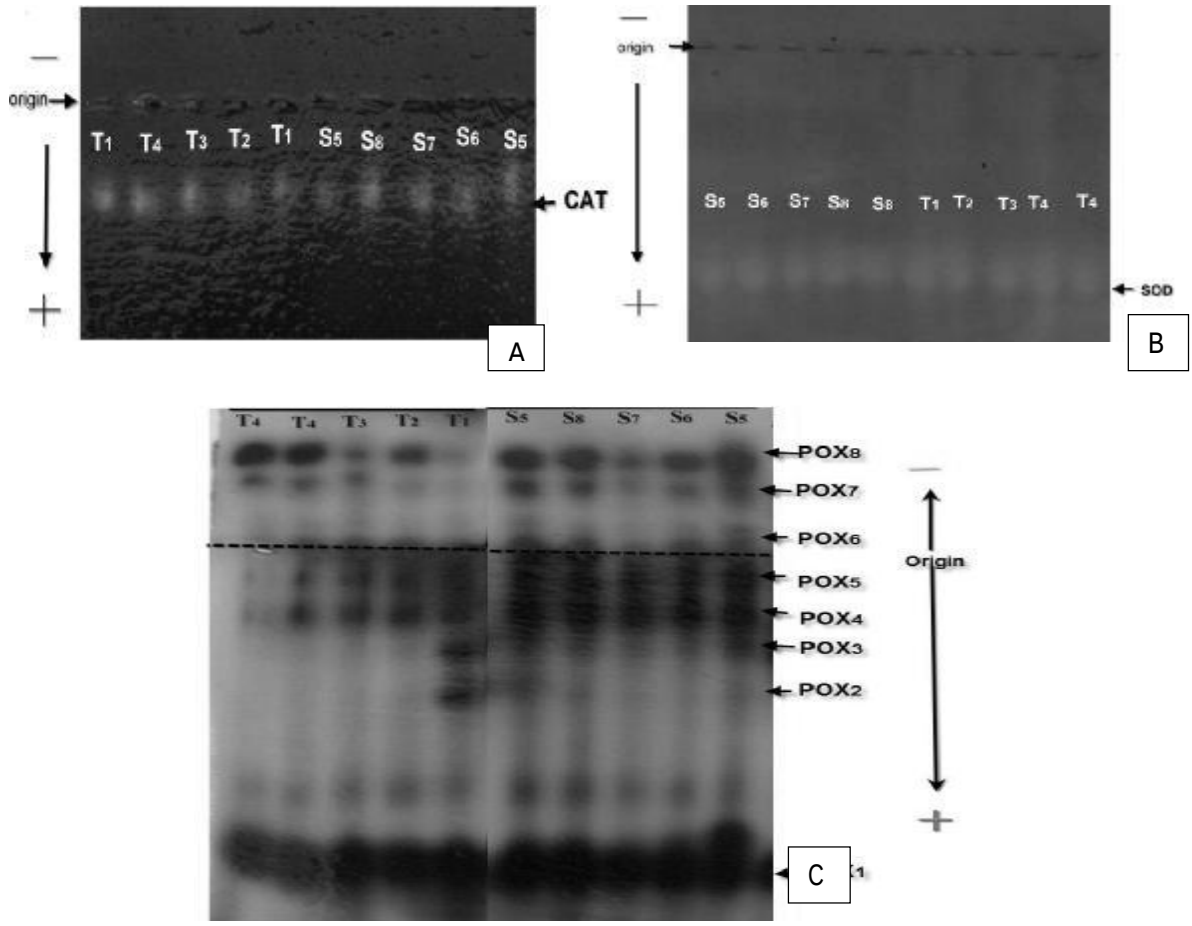

Figure 1. Isozyme profiles of eight wheat genotypes; Catalase (A), Superoxide dismutase (B), Peroxidase (C) (T: Tolerant, S: Sensitive).

A large decrease was observed for SOD activity in the seedling roots under drought stress in comparison to the control (Table 2). CAT activity in the normal and stress conditions did not change significantly, while it was not the same in the varieties during drought stress. Activity of CAT in T_4 was significantly greater than that of other genotypes ($P<0.001$, data are not shown).

As shown in Table 3, there was significant difference between drought tolerant and drought sensitive groups. The drought tolerant group showed a significantly increased CAT activity (40.95%) as compared to the sensitive one (28.68%). No significant interactions were found between groups and stress conditions for SOD and CAT isozyme activities (data not shown)

Table 2. Densitometric activities of catalase, peroxidase and superoxide dismutase isozymes in winter wheat seedling roots at different water stress conditions

Drought treatment	Catalase	Peroxidase				Superoxide dismutase
		POX_{Total}	POX_1	POX_3	POX_6	
Water stress	18.33a	0.183a	0.480a	0.094b	0.072b	32.10b
Normal	18.72a	0.174a	0.316b	0.143a	0.106a	35.91a

In each column, the values with the same letters are not significantly different at $p<0.05$.

Table 3. Densitometric activities of catalase, peroxidase and superoxide dismutase isozymes in winter wheat seedling roots in drought sensitive and tolerant groups

Tolerance	Catalase	Peroxidase				Superoxide dismutase
		POX_{Total}	POX_3	POX_4	POX_5	
Tolerant	40.95a	0.181a	0.094b	0.190b	0.197b	41.28a
Sensitive	28.68b	0.192a	0.143a	0.267a	0.264a	40.56a

In each column, the values with the same letters are not significantly different at $p<0.05$.

Among POX isoforms there were significant differences in activity of POX_1, POX_3 and POX_6 between stress and normal conditions (Figure 2). Subsequent drought stress imposition resulted in no significant change in total POX. Total POX (POX_{Total}) content in stress and normal conditions was similar (Table 2). Tolerant plants roots exhibited more decrease in POX level particularly in POX_3, POX_4 and POX_5 than sensitive plants during water stress (Figure 3). As shown in Table 2, from isoforms of POX, only POX_1, POX_6 and POX_3 showed significant difference between water stress and normal condition. POX_1 exhibited higher activity in both environments (Figure 2). Stress treatment resulted in a significant increase in POX_1 activity by about 31.1% compared to the control. In contrast, the roots in the normal condition showed significant increase in POX_3 and POX_6 as compared to the stress condition (Figure 2). The roots in the normal environment exhibited higher POX_3 and POX_6 activity by about 34.2% and 32%, whereas drought-stressed showed no change in total POX activity by water stress. The activity of POX_3 was highest in the sensitive genotype S_8 and was lowest in the tolerant genotypes T_2, T_3 and T_4 (data are not shown).

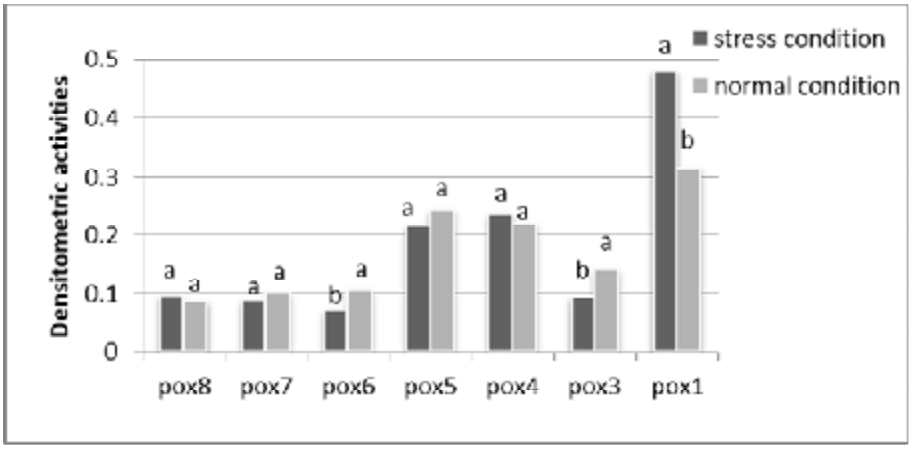

Figure 2. Changes in densitometric activities of peroxidase isoforms in roots at water stress and normal conditions

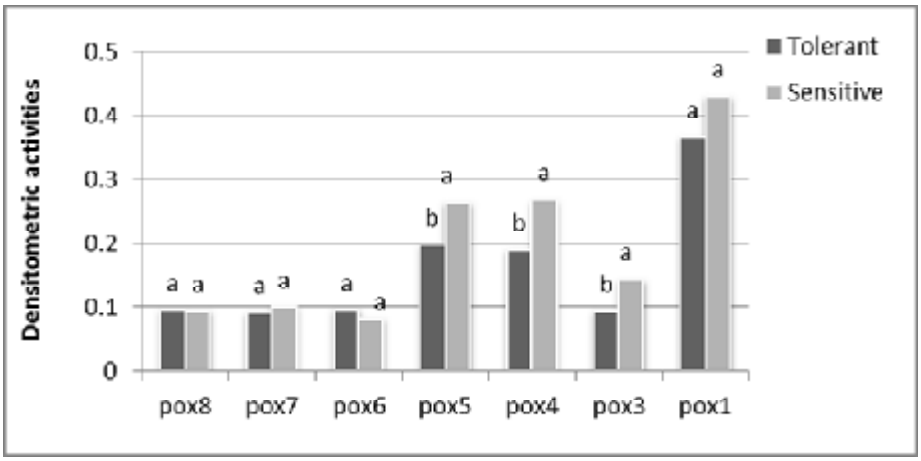

Figure 3. Changes in densitometric activities of peroxidase isoforms in roots of sensitive and tolerant genotypes under water stress

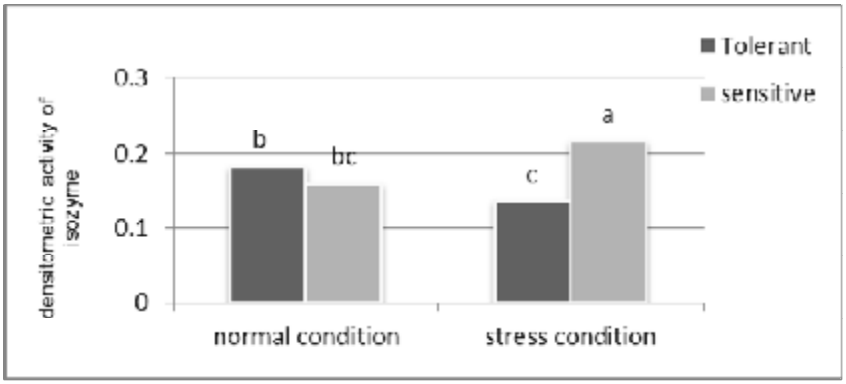

Figure 4. Changes in densitometric activities of peroxidase isoforms in roots of sensitive and tolerant genotypes at normal and water stress condition

For POX, the interaction of groups (sensitive and tolerant) with drought treatments was significant ($P<0.001$) (Figure 4). This interaction indicated that POX activity in the sensitive group of wheat genotypes was significantly higher than the tolerant group at the stress condition, while in the normal condition it was in opposite direction but insignificant.

Discussion

The results showed significant decrease of SOD activity in roots of seedlings from mother plants that were exposed to water stress. The ability of plants to overcome oxidative stress partly relies on the induction of SOD activity and subsequently on the up-regulation of other downstream antioxidant enzymes (Alscher *et al*. 2002). According to the fact that SOD processing is known to be substrate inducible (Tsang *et al*. 1991), a decrease in the SOD activity may be attributed to the decreased production of active oxygen species as substrate that leads to decreased expression of genes encoding. Under drought stress, enhanced SOD activity was found in pea (Moran *et al*. 1994), and tobacco (Van Rensburg and Kru¨ger 1994), decreased SOD activity in sunflower seedlings (Quartacci and Navaro 1992), unaffected SOD activity in maize (Luna *et al*. 1985), while in wheat, SOD activity increased or remained unchanged in the early phase of drought but decreased with prolonged water stress (Zhang and Kirkham 1995). According to Zhang and Kirkham (1995), considering drought stress period prolongation in our study, decrease in SOD activity was not unexpected.

In terms of our results, although the activities of SOD in seedling roots were decreased by drought stress, CAT activity remained unchanged in all experimental genotypes. Similar results were achieved in the water-stressed wheat plant (Bartoli *et al*. 1999). Reports on catalase activity under drought stress are also heterogeneous. CAT activity has been shown to increase (Mittler and Zilinskas 1994; Jiang and Zhang 2002) and also to remain unchanged or even decrease under water stress (Fu and Huang 2001; Turkan *et al*. 2005). Luna *et al*. (1985) suggested that CAT is a less susceptible scavenging enzyme than POX regarding oxidative stress. CAT has in fact a lower affinity for H_2O_2 than POX which suggests its role in counteracting excessive H_2O_2 production. Furthermore, excess H_2O_2 may attack and inhibit POX, hence CAT activity is likely to be favorable in maintaining POX activity under severe drought stress (Cruz de Carvalho 2008).

Sensitive wheat genotypes showed a significant decrease in SOD activity by about 10%. This may be related to the low potential of these genotypes to remove $O2^{-}$ under water deficit. H_2O_2, which resulted from the action of SOD, is toxic to cells. Therefore, it is important that H_2O_2 be scavenged rapidly by the antioxidative defense system to water and oxygen (Guo *et al*. 2006).

The present study indicates that POX and SOD remained unchanged in different drought tolerant genotypes, even though there were significant difference among activities of POX isoforms. Similar results were achieved by Csiszar *et al*.

(2008) in wheat. Isozymes activities changed to less extent but in different manner.

In conclusion, decrease of SOD and POX isoforms activities and lack of change of total POX and CAT activities in the roots of wheat genotypes indicated that the changes of SOD, POX and CAT were not consistent, suggesting possible interactions and synergic effects among these enzymes under drought stress. Since the change of antioxidant enzyme activities are correlated with the abiotic stress tolerance of wheat genotypes, our results suggest that the antioxidant defense capacity and the change of individual enzymatic activities during stress were dependent on the plant genotype. In other words, different wheat genotypes responded differently to water deficiency in terms of the activities of POX and CAT. These results may be used as practical biochemical parameters for selection of drought tolerant wheat genotypes when selecting drought tolerant genotypes for breeding in arid regions.

The analysis of individual isozymes is important, because it can help to understand how the water stress may affect the different subcellular compartments (Bowler *et al*. 1992). After the native polyacrylamide gel electrophoresis (PAGE) analysis, we identified up to eight POX distinct isoenzymes in wheat displaying different isozymic behavior. Therefore, native PAGE along with spectrophotometric analysis could be regarded as a useful tools for this kind of studies.

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