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## Evaluation of the relation of antioxidant enzymes activity with drought tolerance in vegetative and reproductive growth stages

Solmaz Shahandeh\*, Ali Akbar Imani and Hossein Shahbazi

Department of Agronomy and Plant Breeding, Ardabil branch, Islamic Azad University, Ardabil, Iran

### ABSTRACT

*In order to evaluation of high yielding and high tolerance genotypes of wheat and activity relation of some antioxidant enzymes with drought tolerance in wheat genotypes, 10 wheat genotypes were cultured in a randomized complete block design with 3 replications under two condition of complete irrigation and drought tolerance in Agriculture and Natural Resources Research Station of Ardabil. The results of morphological study in this research indicate a high diversity of studied genotypes which high genetic diversity in different corrective actions (classic and traditional) can be useful. Drought stress decreased all traits, especially seed yield and number of fertile tillers. Based on the results of mean comparison and sensibility and drought tolerance indexes of genotypes 2 (C-80-4), 3 (Bloudan/3/Bb/7C\*2//Y50E/3\*Kal/4/MV17) and 4 (Yan7578 128/4 /Chil/24\*Star) chosen as high yielding and high tolerance genotypes. In drought stress conditions, grain yield indicated significant correlation with grain weight per spike, days to flowering, days to maturity, seed weight, harvest index and STI, GMP and MP indexes and also showed negative and significant correlation with non-fertile tiller number, biological yield and sensibility indexes of TOL and SSL. In normal conditions, yield showed positive and significant correlation with its components and indexes STI and GMP, but did not show significant correlation with index trait. The interaction of stages (vegetative and reproductive) was significant at the level of 1% for the enzyme activity of Catalase, Ascorbate peroxidase and Superoxide Dismutase. There was also a significant difference at level of 1% between genotypes in terms of three enzymes activity. There was significant difference at the level of 1% between the interaction of stages in the genotype in terms of enzymes activity of ascorbate peroxides and superoxide dismutase. The enzyme activity of Catalase and superoxide dismutase increased in the reproductive stage than vegetative stage. According to results of principal component analysis and cluster analysis, second group include genotypes 2 (C-80-4), 3 (Bloudan/3/Bb/7C\*2//Y50E/3\*Kal/4/MV17) and 4 (Yan7578 128/4 /Chil/24\*Star) which was more valuable in terms of all traits that evaluated except enzyme activity of catalase and ascorbate and were in the first rank in terms of yield in stress and normal conditions and stress tolerance and were recognized as a superior group.*

**Keywords:** antioxidants, bread wheat, vegetative and reproductive phase, drought stress

### INTRODUCTION

Drought is a major environmental stress that affects different stages of plant growth such as germination stage, seedling establishment and crop production around the world [1, 2]. In recent years, drought is very severe due to changes in climate and atmospheric CO<sub>2</sub> levels. So, the identification of plant varieties resistant to drought is a necessity. Investigation the mechanism that enables plants to find a compromise with drought stress and maintain their growth under those conditions, finally, can help in selection of stress-resistant plants for cultivation in arid and

semi-arid. Under drought stress, the plant photochemical activities are inhibited, chlorophyll content varies and Calvin cycle enzymes activity reduces in the process of photosynthesis [3]. In many plant species, reduction in crop production under drought stress is often related to a decrease in photosynthetic capacity [1]. Drought stress also increase producing of reactive oxygen species (ROS) such as hydrogen peroxide ( $O_2H_2$ ) and superoxide radicals ( $O_2^-$ ) and Hydroxyl (OH), which their accumulation in cells can induce oxidative stress [4]. In the absence of any protective mechanism, ROS can cause oxidative damage of lipids, proteins and nucleic acids disrupt in cells normal metabolism and damage cell membrane which ultimately leads to cell death [5]. Plants have an antioxidant system that control excessive production of active oxygen species under stress and therefore protect them against the harmful effects of active oxygen species and preserve the appropriate level of ROS for growth and message transfer pathways [6]. The antioxidant defense system includes antioxidants with low molecular weight such as, Such as  $\beta$ -carotene, ascorbic acid,  $\alpha$ -Tokufrun and revived glutathione (HSG) plus antioxidant enzymes, including catalase, ascorbate peroxidase, glutathione reductase and peroxidase Gayacol [7]. Antioxidant response is an important process for protecting plants against oxidative damage that is caused by a large part of environmental stresses, including salinity, drought, heavy metals and cold [4]. Drought resistance in crops is associated with the ability to trap ROS and reducing of their harmful effects. The link between increasing of antioxidant enzyme activity and rising plant species resistance under environmental stress has been confirmed in several plant species such as rice [8]. The aim of this study was evaluation of the relation scales of antioxidant enzymes activity with drought tolerance in vegetative and reproductive growth stages.

### MATERIALS AND METHODS

Greenhouse experiment was conducted in a completely randomized design with three replications in two phases: vegetative and reproductive. In greenhouse conditions (experiment II) is sampled of the flag leaf in vegetative and reproductive plants (Before and after flowering), and extraction and activity of 3 enzymes ascorbate peroxidase, catalase and superoxide dismutase were measured: Extraction and measurement of antioxidant enzymes: Leaf tissue in a mortar containing liquid nitrogen was powdered and then enzyme extraction were performed by Sairam et al method [9]. Measurements were performed in both vegetative and reproductive stages. Enzymes activity of superoxide dismutase in the Giannopolitis method and Ries [10], chance and Maehli [11], Ascorbate peroxidase were measured by method of Nakano and Asada [12].

#### *Data analysis*

The results of wheat genotypes comparison were analyzed at vegetative and reproductive stages after doing skew test and strain in a completely randomized design with three replications. Comparison of treatments means was performed using Duncan at 5% level and to examine the relationship between traits and the estimated indexes, Simple correlation coefficients were estimated. To analyze information and drawing shapes were used of software SPSS, SAS and EXCEL.

### RESULTS AND DISCUSSION

For more information about the effect of growth stages on the characteristics change process and also the interaction of growth stage with other factors, the time factor has been entered the research and variance analysis was performed factorial. Results of variance analysis are presented in two stages (Table 1). The effect of stages (vegetative and reproductive) for the enzymes activity of ascorbate peroxidase, catalase and Superoxide dismutase was significant at the 1% level. There were significant differences between the genotypes in terms of activities of all three enzymes at the level of 1%. The interaction of stage in genotype in terms of enzyme activity of ascorbate peroxidase, catalase and Superoxide dismutase was significant at the level of 1%; it means that genotypes had not same reaction in both the vegetative and reproductive stages, in terms of ascorbate peroxidase and superoxide dismutase activity. This means that the reaction of characteristics was different at two stages of vegetative and reproductive. However, the interaction effect of genotype on the rate of catalase activity was non-significant. The mean of physiological traits at different stages (vegetative - reproductive) are presented in Table 2. Results showed that the activity rate of all three enzymes catalase and superoxide dismutase increased in the reproductive stage than vegetative stage.

Similar results have been reported on this issue, including Sairam et al [13], who were investigated four varieties of wheat under drought stress in a pot.

In this experiment, the enzyme superoxide activity Dismutase, catalase, ascorbate peroxidase increased under stress. Shahbazi [14], also found similar results in a pot experiment. In the study, Jiang and Huang [15], drought stress in the activity of three enzymes catalase and superoxide dismutase and ascorbate peroxidase increased after 6 to 12 days after starting tensions, but their activity was decreased with stress. The initial increase in the activity of these enzymes can be caused by excessive accumulation of reactive oxygen species in the starting stress [15]. Shao et al showed that reaching maturity and aging, decreased the activity of peroxides and catalase enzymes [16]. There are many reports on the increase, decrease or no effect of dry on CAT activity in references [17, 18, and 19]. In the view of dat et al [20] the reduction of catalase activity under drought stress can be due to light inactivation of this enzyme and prevention of its synthesis in the dark. In the research of Luna et al [21] also was decreased catalase gene expression under stress which is inconsistent with the results. But Ping et al [22] in the effect of stress on corn found that severe stress reduced activities of ascorbate peroxidase, catalase and superoxide dismutase in the reproductive phase and increased lipid peroxidation in all phases. The study results of Jiang and Huang [16] showed that severe stress can weaken removal systems of  $o_2^-$ . Reducing of enzyme activity under stress can reduce the rate of synthesis or increase degradation of these enzymes. The average of studied genotypes in terms of catalase activity in both vegetative and reproductive stages (Figure 1) showed that all genotypes had high activity in the reproductive stage than vegetative stage, genotypes 5, 1 and 2 in reproductive stage and genotypes 1 and 6 in vegetative stage had the highest activity. The average of studied genotypes in terms of Scorbutic peroxides activity in both vegetative and reproductive stages (Figure 2) showed that all genotypes had high activity in the reproductive stage than vegetative stage, genotypes 4, 6, 10 and 5 had high activity in the vegetative stage. The interaction of genotypes in stage in terms of activity scale of enzyme SOD was significant at level of 1%, the average of studied genotypes in terms of activity scale of enzyme SOD in both vegetative and reproductive stages ( Figure 3) showed that all the studied genotypes had high activity in the reproductive stage than vegetative stage, genotypes 3, 1 and 10 in reproductive stage and genotype 6 in vegetative stage had high activity.

**Table 1. Results of variance analysis of antioxidant enzymes in both reproductive and vegetative stages in the studied genotypes**

s.v	FD	Catalase	Ascorbate	SOD
stage	1	16.95**	6854.2**	1290.66**
G	9	1.74**	374.13**	801.19**
G*S	9	0.31 <sup>ns</sup>	184.99**	412.84**
E	40	0.22	23.79	17.84
C.V		25.19%	10.76%	23.28%

\*\*\* And <sup>ns</sup> respectively show Significant at 1% level and non significant

**Table 2. The mean comparison of antioxidant enzymes levels in both vegetative and reproductive**

Stage	Catalase	Ascorbate	SOD
vegetative	1.314	34.652	b 13.509
reproductive	2.377 a	56.028	a 22.765 a

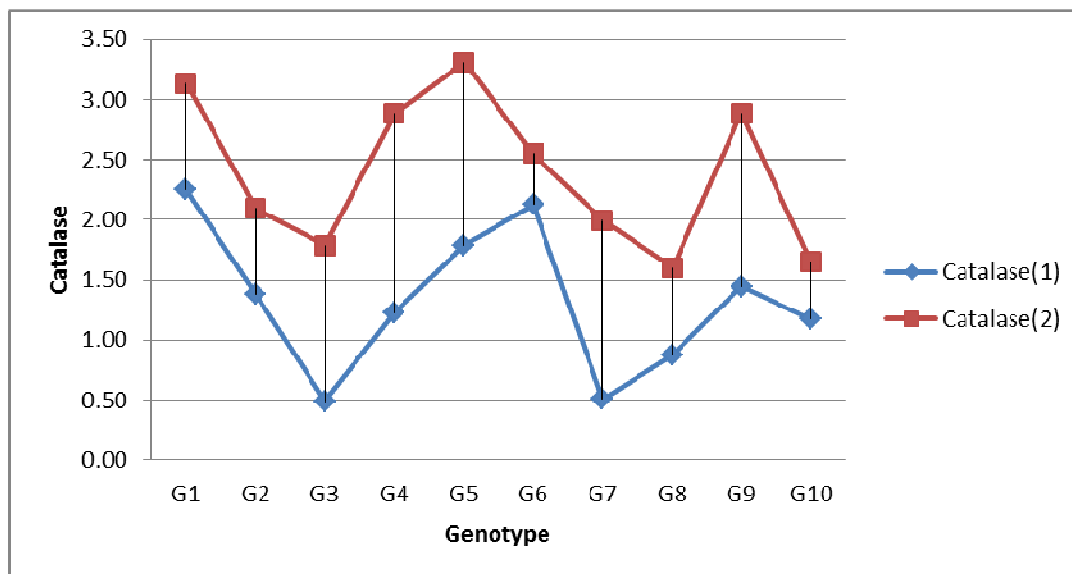


Figure 1. The activity of catalase in the separation of genotypes studied in the vegetative and reproductive stage

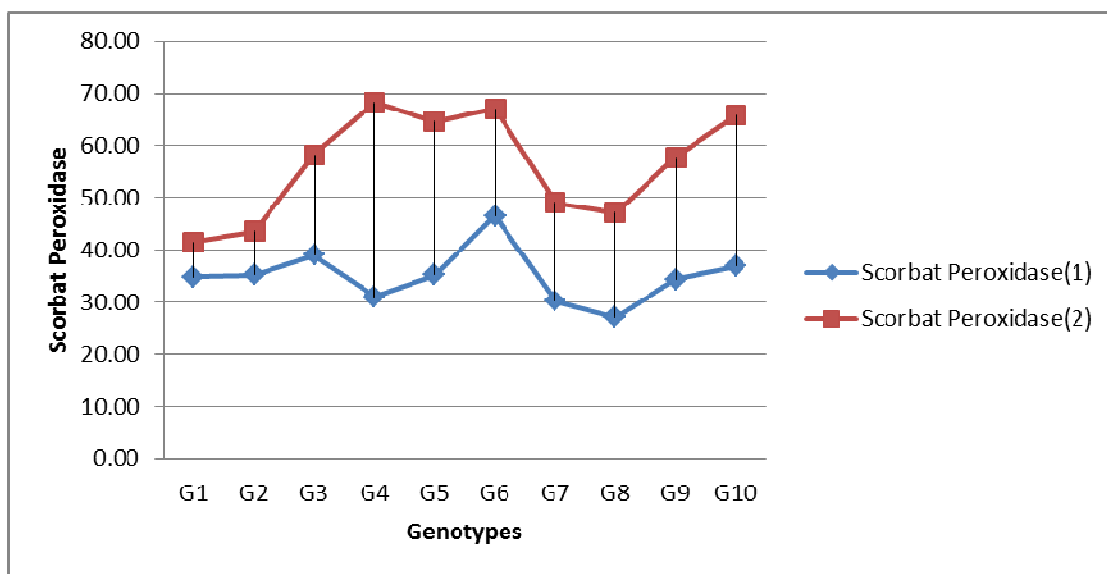


Figure 2. The activity of Ascorbate peroxidase in the separation of genotypes studied in both vegetative and reproductive stage

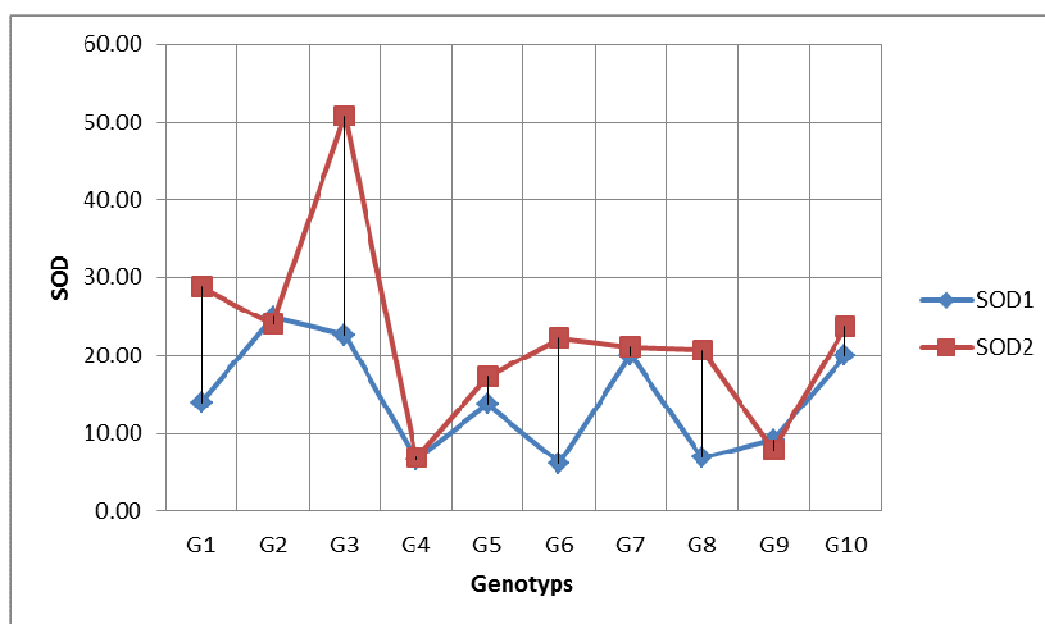


Figure 3. The activity of superoxide dismutase (SOD) in the separation of genotypes studied in both vegetative and reproductive stage

### CONCLUSION

The effect of stages (vegetative and reproductive) for the enzymes activity of scorbutic peroxidase, catalase and Superoxide dismutase was significant at 1% level. There were significant differences between the genotypes in terms of activities of all three enzymes at the level of 1%. The interaction of stage in genotype in terms of enzyme activity of scorbutic peroxidases, catalase and Superoxide dismutase was significant at the level of 1%. The activity rate of all three enzymes catalase and superoxide dismutase increased in the reproductive stage than vegetative stage.

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