

Antioxidant Response of Lentil Genotypes (*Lens culinaris* Madke) to Drought Stress in Different Reproductive Growth Stages

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ABSTRACT

In order to investigate the relationship between some physiological traits with drought tolerance in lens cultivars, 10 advance cultivars of lens, were planted in randomized complete design with 3 replications under non-stress and terminal drought stress conditions in research station of Islamic Azad University, Ardabil, Iran in 2012. Leaf tissues at reproductive stage (10, 20 and 30 day after reproductive stage) were ground to fine powder in liquid nitrogen and then enzyme extraction was done according to Sairam. The activity of superoxide dismutase (SOD) was measured by the method of Giannopolities and Ries. Catalase (CAT) was assayed by the method of Chance and Maehly. The assay of ascorbate peroxidase (APX) activity was performed as described by Nakano and Asada. The enzyme activities were expressed in terms of specific activity, Results of variance analysis are presented in two Conditions, tree stage and 10 genotypes showed The effect of Non-stress and drought stress conditions for the enzymes activity of catalase, ascorbate peroxidase, and Superoxide dismutase was non-significant, stages (10, 20 and 30 day after reproductive stage) for the enzymes activity of ascorbate peroxidase, catalase and Superoxide dismutase was significant at the 5% level. There were significant differences between the genotypes in terms of activities of all three enzymes at the level of 1%. Stress \times stage and Stress \times Genotype interactions also was significant in all cases. Genotype \times stage and Stress \times Genotype \times stage interactions also was not significant in all cases. The mean comparison of physiological traits showed that the highest Catalase and ascorbate peroxidase enzymes activity were related to the stage of 20 days after flowering in two Conditions (non-stress and drought stress conditions). And genotypes mean comparison results on the Catalase enzyme activity that genotype No.2 had the highest values in tow enzymes. Results showed that the activity rate of increased in the reproductive stage at drought stress condition. And that genotypes No.2 and 3 had highest SOD enzyme activity at drought stress condition.

KEYWORDS: Drought, Heritability, physiological trait, reproductive growth stages.

1. INTRODUCTION

Biological stress is defined as any disadvantageous condition that reduces or Inhibits the normal growth and/or survival of biological organisms such as plants [Jones et. al., 1989]. Being sessile, the plants are vulnerable to environmental stresses, which destroy the normal growth cycle and reduce the yield. Mahajan and Tuteja divides environmental stress into two main groups as biotic and abiotic [Mahajan and Tuteja, 2005]. Biotic stresses are pathogens, insects, herbivores and Rodents, whereas low and high temperatures, salinity, drought, excess water, Radiation, heavy metals, pesticides, ozone, wind, nutrient deprivation in soil are Some examples to abiotic stresses. Drought stress has the highest percentage with 26%. It is followed by mineral stress with 20%, cold and freezing stress with 15%. All the other stresses get 29% whereas only 10% area is not exposed to any stress factors [Blum, 1986]. A free radical is any species capable of independent existence that contains one or more unpaired electrons [Halliwell and Gutteridge, 2006]. An unpaired electron is one that occupies an atomic or molecular orbital itself. The simplest free radical is the atomic hydrogen. There are many types of free radicals such as oxygen and nitrogen radicals in living systems. Oxygen levels in atmosphere elevated over 2.2 billion years ago by activities of Cyanobacteria. They used energy of sun to split water into hydrogen and oxygen atoms by means of photosynthesis. Since then, hydrogen atoms have been used as reducing agents while oxygen has released into the atmosphere as a by-product [Lane, 2002]. Ever since the introduction of molecular oxygen (O₂) into the atmosphere, reactive oxygen species (ROS) have been the unwelcome Companions of aerobic life [Mittler et al., 2004]. Although molecular oxygen (O₂) is relatively unreactive due to its electron configuration [Eltner, 1987], it is thermodynamically a potent oxidizing agent. When O₂ tries to oxidize a no radical by accepting a pair of electrons from it, both these electrons must have the same spin to fit into the vacant spaces in the uppermost free orbitals. A pair of electrons in an atomic or molecular orbital cannot meet this criterion since they have opposite spins. This spin restriction makes O₂ prefer to accept its electrons once at a time [Halliwell, 2006]. Ground state oxygen may be converted to the much more reactive ROS forms either by energy transfer or by electron transfer reactions. The former leads to the formation of singlet oxygen, whereas the latter results in the sequential reduction to superoxide, hydrogen peroxide, and

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hydroxyl radical [Apel, Hirt, 2004]. Higher plants have several antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) and some low molecules of non-enzyme antioxidants [Greene, 2002]. Which can confer resistance to plants due to scavenging reactive oxygen species. 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. The present paper reports the variation in and association of physiological traits with yield of lentil cultivars under non-stress and drought stress at reproductive stage.

MATERIALS AND METHODS

10 advance cultivars of lens were planted in randomized complete design with 3 replications under non-stress and terminal drought stress conditions in research station of Islamic Azad University, Ardabil, Iran in 2012. Leaf tissues at reproductive stage (10, 20 and 30 day after reproductive stage) were ground to fine powder in liquid nitrogen and then enzyme extraction was done according to Sairam *et al.*, [Sairam, *et al.*, 1998]. The activity of superoxide dismutase (SOD) was measured by the method of Giannopolities and Ries [Giannopolities and Ries, 1977]. Catalase (CAT) was assayed by the method of Chance and Maehly [Chance and Maehly, 1955]. The assay of ascorbate peroxidase (APX) activity was performed as described by Nakano and Asada [Nakano and Asada, 1981]. The enzyme activities were expressed in terms of specific activity (Unit/mg Fresh Weight).

Statistical analysis

Statistical analysis was performed using combined ANOVA for yield (in non-stress and drought stress conditions) and combined ANOVA (In stages and drought conditions) for activity of antioxidant activity. Means compared with the Duncan's multiple range tests. Correlation coefficients were used for investigating the relation of traits. Statistical analysis was carried out with the SAS 9.2 package.

RESULTS AND DISCUSSION

Results of variance analysis are presented in two Conditions, tree stage and 10 genotypes showed (Table 1). The effect of Non-stress and drought stress conditions for the enzymes activity of catalase, ascorbate peroxidase, and Superoxide dismutase was non-significant, stages (10, 20 and 30 day after reproductive stage) for the enzymes activity of ascorbate peroxidase, catalase and Superoxide dismutase was significant at the 5% level. There were significant differences between the genotypes in terms of activities of all three enzymes at the level of 1%. Stress \times stage and Stress \times Genotype interactions also was significant in all cases. Genotype \times stage and Stress \times Genotype \times stage interactions also was not significant in all cases. The mean comparison of physiological traits showed that the highest Catalase and ascorbate peroxidase enzymes activity were related to the stage of 20 days after flowering in two Conditions (non-stress and drought stress conditions) (Figures 1 and 2). And genotypes mean comparison results on the Catalase enzyme activity that genotype No.2 had the highest values in tow enzymes (Figure 2 and 4). Results showed that the activity rate of increased in the reproductive stage at drought stress condition (Figures 5). And that genotypes No.2 and 3 had highest SOD enzyme activity at drought stress condition (Figures 6). Jiang and Huang [Jiang and Hung, 2001], 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 [Jiang and Hung, 2001]. Shao *et al.* showed that reaching maturity and aging, decreased the activity of peroxides and catalase enzymes [shao *et al.*, 2005]. There are many reports on the increase, decrease or no effect of dry on CAT activity in references [Zhang and Kirham, 1994; shao *et al.*, 2005; Smirnoff, 1993]. In the view of dat *et al.* [Dat *et al.*, 1998] 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.* [Dat *et al.*, 1998] also was decreased catalase gene expression under stress which is inconsistent with the results. But Ping *et al.* [Luna. *al.*, 2004] 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 [shao *et al.*, 2005] 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.

Table 1. Mean squares in analysis of variance for antioxidant enzymes activity

Source of Variation	df	Mean squares		
		Catalase	SOD	Ascorbate
Stress	1	Ns	ns	ns
Stage	2	*	*	*
Genotype	9	**	**	**
Stress × stage	2	*	*	*
Stress × Genotype	9	**	**	*
Genotype × stage	18	Ns	ns	ns
Stress × Genotype × stage	18	Ns	ns	ns
Errore	132			

ns, * and ** indicated not significant, significant at 5% and significant at 1%, respectively

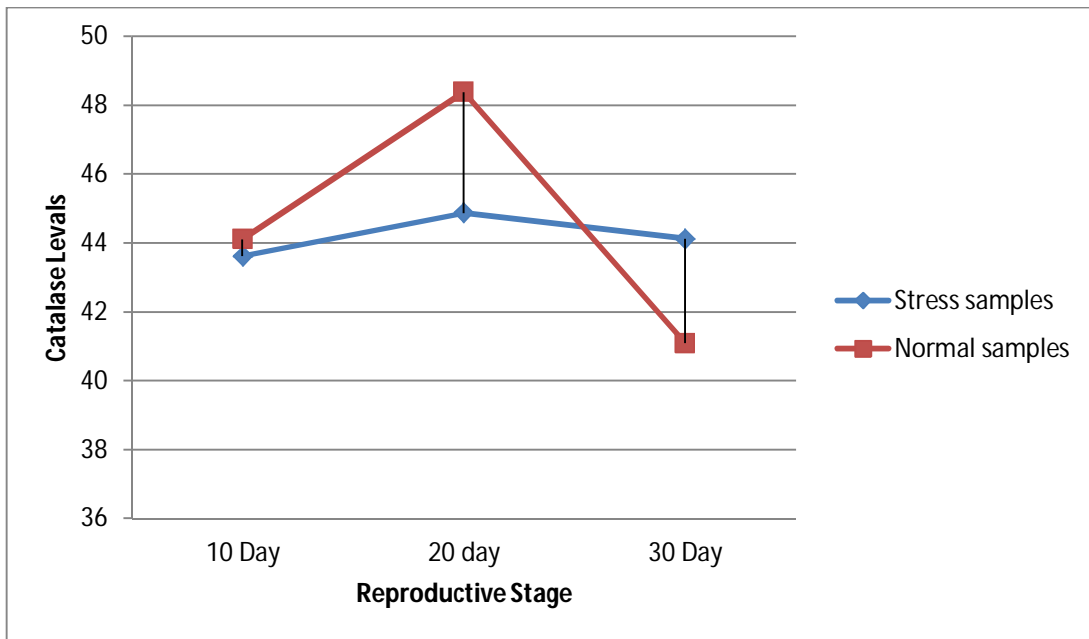


Figure 1. The activity of catalase in non-stress and drought stress conditions at reproductive stage

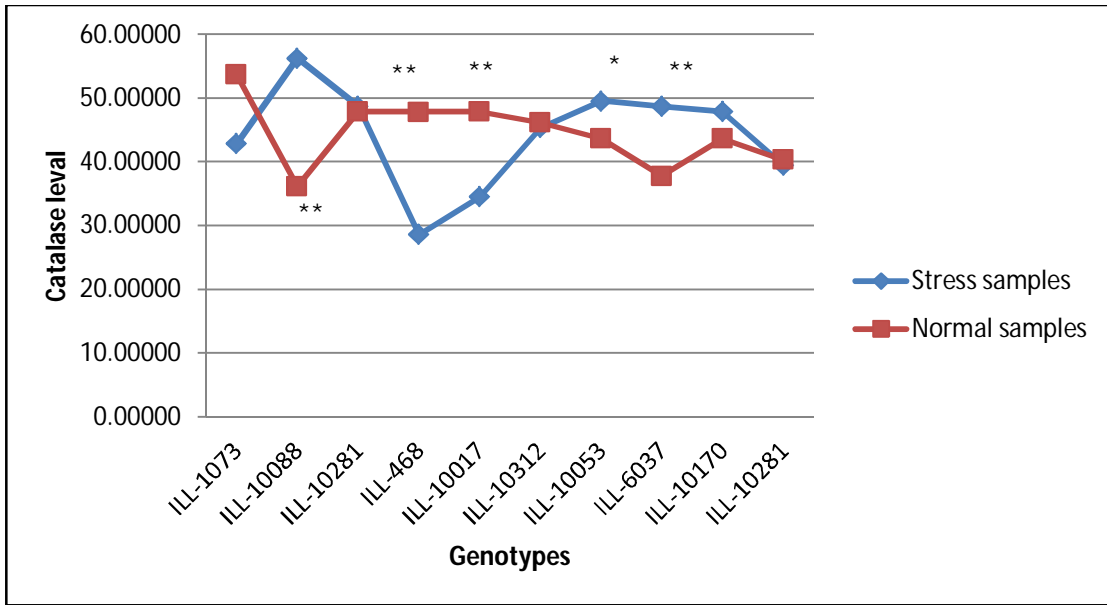


Figure 2. The activity of catalase in of genotypes studied at non-stress and drought stress conditions

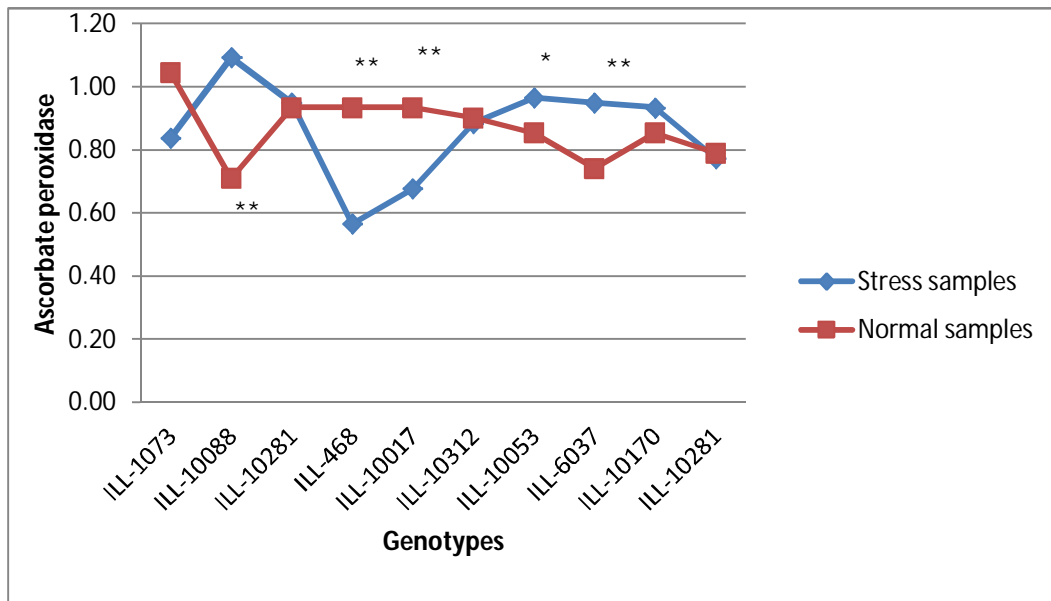


Figure 3. The activity of Ascorbat Peroxidase in non-stress and drought stress conditions at reproductive stage

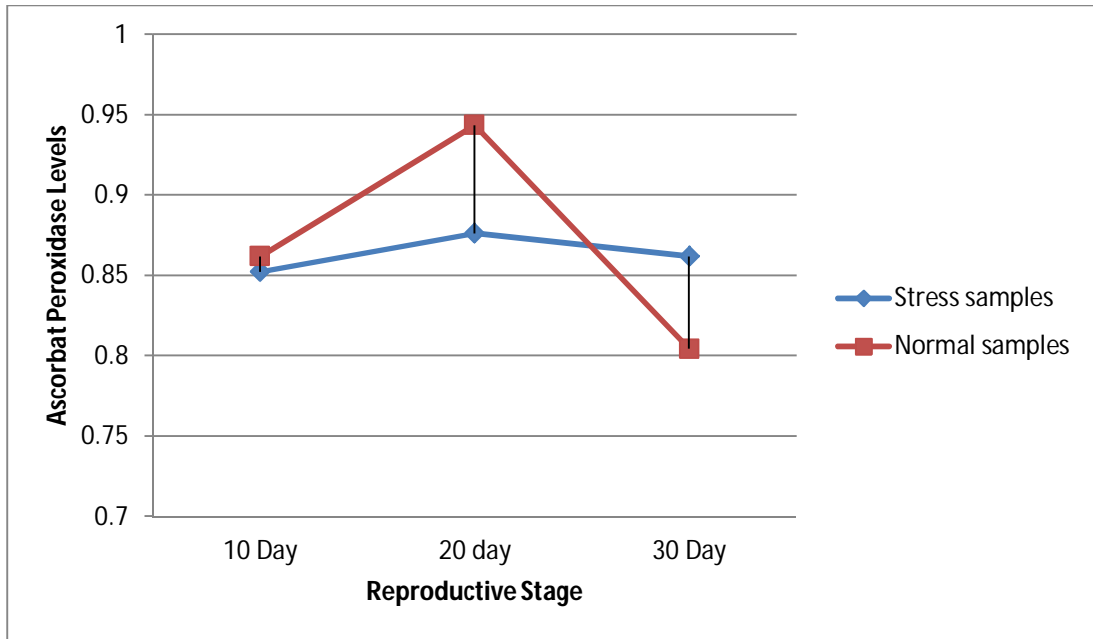


Figure 4. The activity of Ascorbat Peroxidase in of genotypes studied at non-stress and drought stress conditions

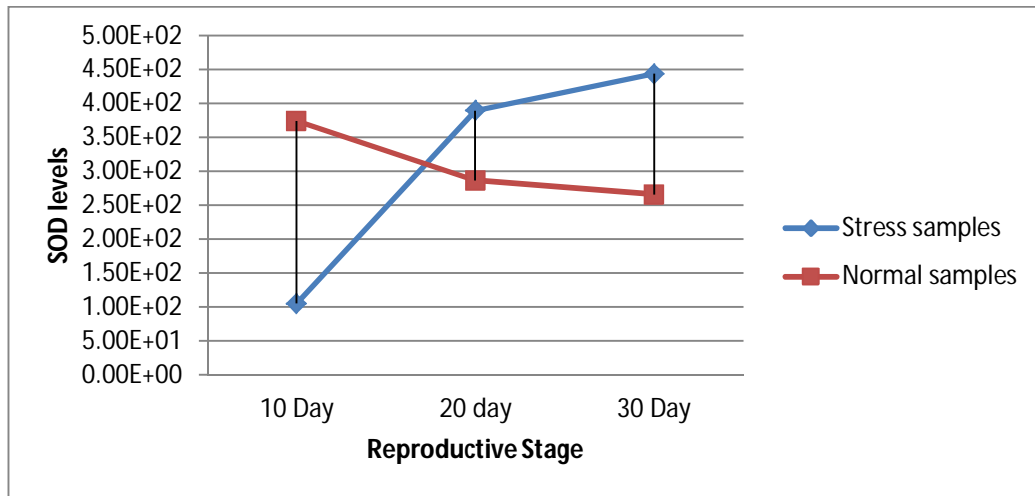


Figure 5. The activity of SOD in non-stress and drought stress conditions at reproductive stage

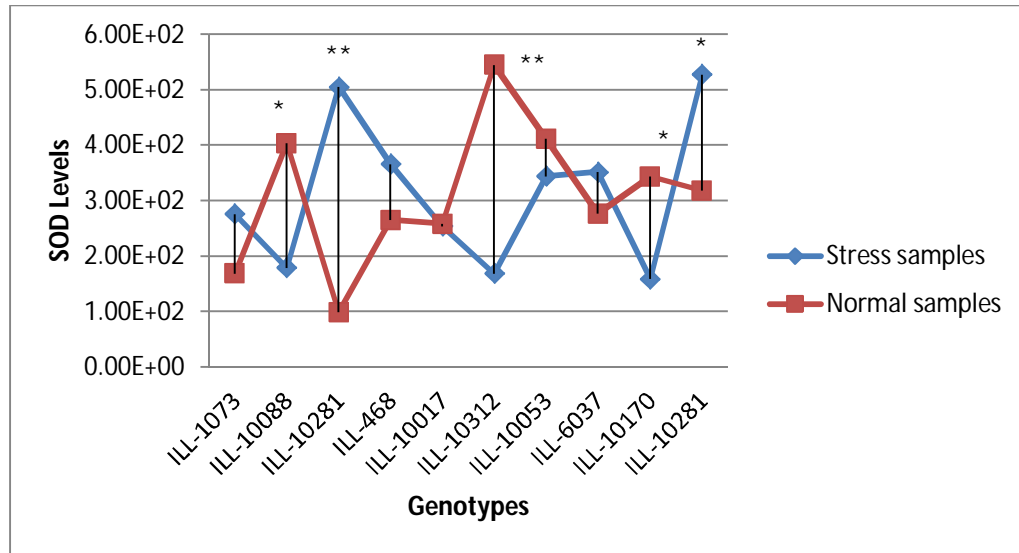


Figure 6. The activity of SOD in of genotypes studied at non-stress and drought stress conditions

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