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Effect of Different levels of NaCl Salinity on Antioxidant Enzyme's Activity in Seedling of Different Wheat Cultivars

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ABSTRACT: Wheat is of paramount importance among cereals and one of the most ancient crops in the globe, so according to Iran's climate condition, it could be concluded that one of the most crucial/limiting stresses which adversely affects this crop is salt stress. Therefore, genotypic evaluation of wheat at a molecular level, while under salt stress, is a mandatory objective. In this regard, the activity of enzymes including catalase (CAT), superoxide dismutase (SOD), and peroxidase (POX) of 14 wheat cultivars during seedling stage was investigated under four salinity treatments, the control (0),weak stress(100mM) moderate stress (200mM), and severe stress (300mM). Quantified dada variance analysis of enzymes activity showed that salt stress has significant effect on activity of some POX isozymes. On the other extreme, among different cultivars of wheat the activity of all detected isoenzymes showed significant differences with the exception of CAT.

Keywords: catalase, peroxidase, salt stress, superoxide dismutase, wheat.

INTRODUCTION

Salinity is one of the primary restricting factors for crop production in both dry and irrigated fields worldwide. In fact, soil and water salinity results in lack of water, Ion toxicity, and lack of nutrients which, gradually, leads to molecular damage, stunted growth, and even plant death (Askari *et al.*, 2006). Meanwhile, salt tolerance in plants is not a constant feature and it may differ in plants, depends on the species and the stage of growth (Sairam & Scerivastava, 2001).

During vegetation growth, crops would always experience different environmental stresses. It is notable that occurrence of environmental stresses cause metabolic disorders in plant cells which probably ends up forming active forms of oxygen (ROS) as one aspect of plant response and cell metabolic disorder to be mentioned (-). As a limiting factor for plant growth and production, oxidative injuries are formed due to harsh environmental conditions (or lack of optimal conditions). Based on prior studies, it is evident that there is a significant correlation between tolerance to environmental-induced oxidative stress and an increase of antioxidant enzymes concentration in plants (Sairam & Scerivastava, 2002). To resist oxidative stress, plants are equipped with a high-efficient defense system which plays a primordial role in plant resistance to drought. In advanced plants the scavenging system by which they neutralize active oxygen, comprises several antioxidant enzymes like Glutathione Peroxidase (GPX), Catalase (CAT), Superoxidase Dismutase (SOD), Ascorbate Peroxidase (APX), and some other enzymes. The antioxidant enzymes protect the membrane from devastative effects of ROSs which are formed due to exerted stress and confer plant stability in tensions (Tan *et al.*, 2006). Therefore, antioxidant enzymes are considered as the swiftest defense units against ROSs oxidative damages (Dirk & Montago, 2002). Plant physiological characteristics including stomata closure, altering the growth regulators pattern, and metabolite accumulation are also other kinds of superior adaptation strategies which plants are acquired to bear stressful conditions (Singh *et al.*, 2004). So, determination of antioxidant enzymes' activity produced under salt stress could accelerate the detection of tolerant root stuck. With regard to this fact that each synthesized compound in cell has a correspondent controlling gene, so in order to pace up production of more resistant plants it would be promising to identify the associated genes and transfer them into the other plants (Sairam & Siriostav, 2002). Hence, selecting appropriate cultivars, predicting the accurate occurrence time of stress, and indicating other Intracellular factors, which guarantee protecting the plant against environmental stresses, are essential for breeding a given crop.

This study carried out aimed at investigating the effect of different levels of salinity during seedling stage on the activity of some antioxidant enzymes and scrutinizing these enzymes in correlation with resistance or susceptibility of different wheat cultivars to drought.

MATERIALS AND METHODS

In this study, 14 cultivars/genotypes of winter wheat have been studied. Samples were provided from Plant Breeding and Biotechnology department of University of Tabriz and Maragheh Dryland Research Institute (Table 1) and then they were planted in a completely randomized factorial design with three replications, applying four different levels of NaCl (0, 100, 200, and 300 mM). In this research, for each genotype about 5 seedlings were taken from each experimental unit (Petri dish) and enzyme extraction from the seedling leaves was performed according to Valizadeh *et al.* (2011). Considering this fact that enzymes are very vulnerable to high temperatures and would be deformed, all extraction stages and loading the gel were performed in the temperature range of 0-4°C. After electrophoresis in polyacrylamide gels (7.5%) and making multilayer gels by means of thing metal wires, then superoxidant dismutase and catalase enzymes were stained based on Solties and Solties (1990) and for staining peroxidase Olson and Warner Method (1993) were employed. After completing the staining, isozyme bands which appeared on the gel were photographed and then scored. After scanning fixed gels, enzymes activity were quantified by MCID 0.7 Analysis evaluation. For this purpose, area and density of each isozyme bands were measured by the software, and then multiplication of area to intensity of the bands (D × A) were considered as isozyme densitometric activity. It should be noted that before statistical analysis, data was tested to make sure whether they are normal and to convert them if necessary.

Table 1: Characteristics of studied winter wheat.

Genotype Code	Drought Response	Name / Pedigree
1	Tolerant	Unknown-1
2	Tolerant	Ghafghaz//F9.10/Maya"s"IRW-92-1-D474-OMA-OMA-OMA-OMA-IMA-OMA
3	Tolerant	Azarbaijan/Roozi-84
4	Tolerant	Tous
5	Tolerant	Azarbaijan / Gobostan
6	Sensitive	FKG13/4/NWT/3/TAST/SPRW//TC198-0139-OAP-OAP-OMAR-5MAR
7	Tolerant	6149-27-1/Sbalan// 84.40023
8	Sensitive	RINA-11
9	Sensitive	Azarbaijan/sartoveskaya-29
10	Sensitive	Cimmyt/ Saysonz
11	Sensitive	JANZ-QT3685 OAUS
12	Tolerant	Azar-2
13	Tolerant	Sardari
14	Tolerant	DARIC95-010-0MA-0MA-0MA-6MA-0MA

RESULTS AND DISCUSSION

Fig. 1 to 3 are examples for banding pattern of antioxidant catalase (CAT), Peroxidase (POX), and superoxide dismutase (SOD) enzymes in some wheat genotypes which were examined under four different levels of salinity. In this research, only one isoform for CAT (Fig. 1), three isoforms for POX (Fig. 2), and two isoforms for SOD (Fig. 3) were detected.

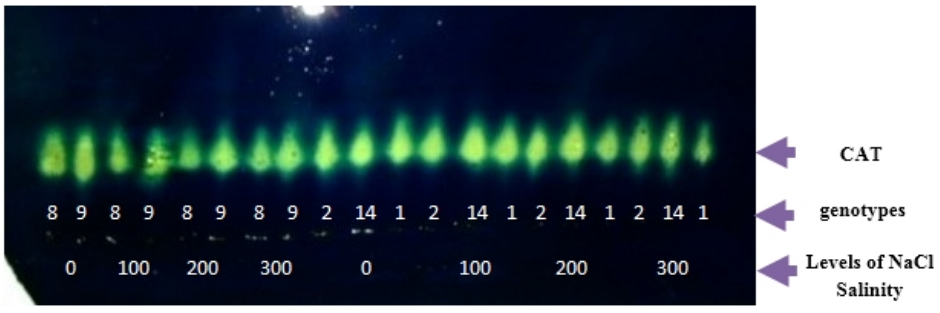

Fig. 1. A banding pattern of Catalase (CAT) isozymes in some wheat genotypes, under salt stress.

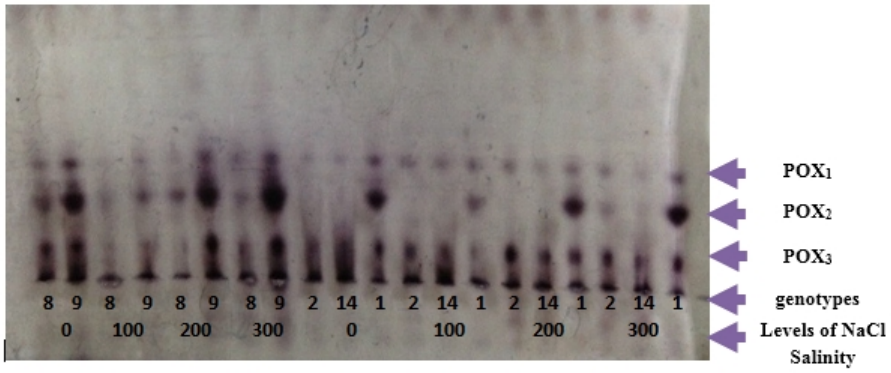

Fig. 2. Banding pattern of Peroxidase (POX) in wheat genotypes, under salt stress.

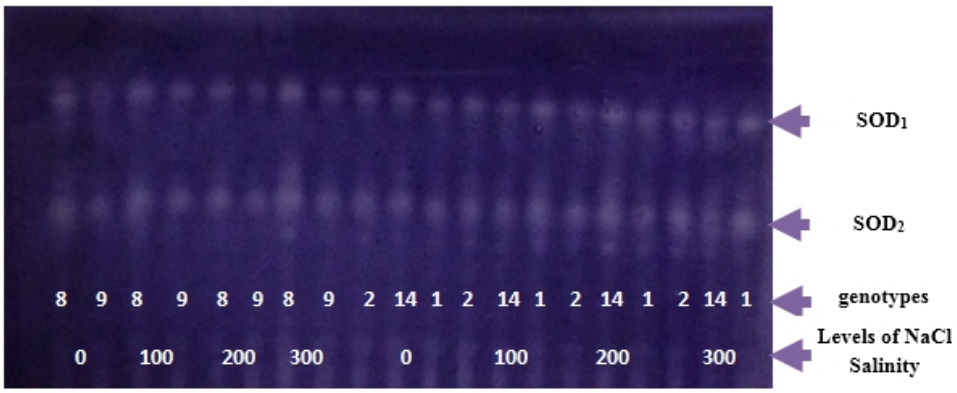

Fig. 3. Banding pattern of superoxide dismutase (SOD) in wheat genotypes, suffering from salt stress.

The results of variance analysis for the effect of salinity and genotype on isozymes activity of three studied enzymes in the leaves of wheat seedlings are given in Table 2. In this experiment, the coefficients of variation for each case were variable, ranging from 29 to 59 percent which represents the effect of different factors in evaluation of antioxidant enzymes activity; therefore, the effect of studied factors in many cases were insignificant. In addition, the interaction of genotype x salinity were not significant for each isozyme which indicates the same reaction of wheat genotypes in different levels of salinity from the enzyme activity point of view. Likewise, there were no significant differences for catalase activity between studied genotypes.

Table 2: Variance analysis of antioxidant enzymes activity in studied wheat genotype seedlings under salt stress.

VS	DF	MS					
		CAT	POX1	POX2	POX3	SOD1	SOD2
Genotype	13	0.00009 ^{ns}	0.00005 **	0.012 **	0.006 **	0.0001 **	0.014 **
Salinity	3	0.00008 ^{ns}	0.00007 ^{ns}	0.007 *	0.009 *	0.001 ^{ns}	0.044 ^{ns}
Genotype× Salinity	39	0.00005 ^{ns}	0.00003 ^{ns}	0.003 ^{ns}	0.002 ^{ns}	0.0001 ^{ns}	0.007 ^{ns}
Error	112	0.0001	0.00002	0.003	0.002	0.0001	0.015
VC (%)		47	49	58	59	29	54

*,**, and ns are significant at (P<5%), significant (P<1%), and insignificant respectively.

As one of the most important enzymes and occupying a very complex regulating system (Lona *et al.,* 2004), CAT is an enzyme which is found in all living organisms like plant cells, animal cells, and aerobic in a way that too many different roles are reported for CAT enzyme in oxidative stress. In previous studies it was reported that CAT activity under abiotic stress (e.g. drought and salinity) would be increased (Mittler & Zylinskas, 1994; Ribo *et al.,* 2002) and decreased (Turkan *et al.,* 2005; Noreen & Ashraf, 2009; Aydin *et al.,* 2011). Regarding CAT enzymatic activity, there was no significant difference between all three isozymes among 14 studied genotypes. However, as for salinity levels, used in this study, only two isozymes (POX2 and POX3) were significant (P<5%) from three detected isoenzymes.

The interaction of salinity x genotype for all POX isozymes was no significant. Mean comparison of POX1 enzyme activity for examined wheat genotypes showed (Fig. 4) that 9 genotype manifests the highest activity mean which had no significant difference with 2, 4, and 5 genotypes; meanwhile, 6 and 12 genotypes occupies the lowest enzyme activity mean among all wheat genotypes.

Considering POX2 isozyme, the highest enzyme activity mean, under different salinity conditions, is associated to 1 and 14 genotypes which statistically showed no significant difference with 2, 6, 8, 9, 12 genotypes; on the other extreme, 3 and 4 genotypes acquired the lowest enzyme activity. It has also been evident that mean comparison results for both POX3 (Fig. 6) and POX1 confirmed that 9 genotype with the same statistical score to 4, 5, 7, and 8 genotypes indicated the highest isozyme activity. Like POX1, the least enzyme activity for this isozyme is belong to 12 genotype which according to the correlation table (Table 4) it could be testified that there is a positive and significant correlation between these two isozymes.

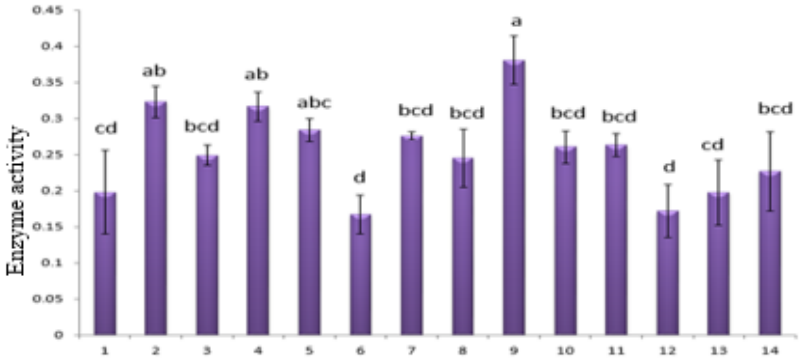

Fig. 4. Densitometric activity mean of POX1 in seedling of 14 wheat genotypes.

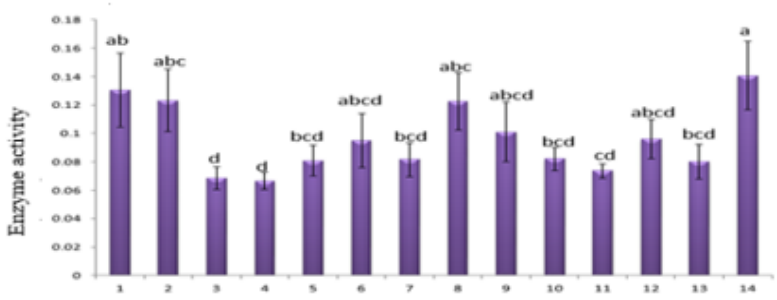

Fig. 5. Densitometric activity mean of POX2 in seedling of 14 wheat genotypes.

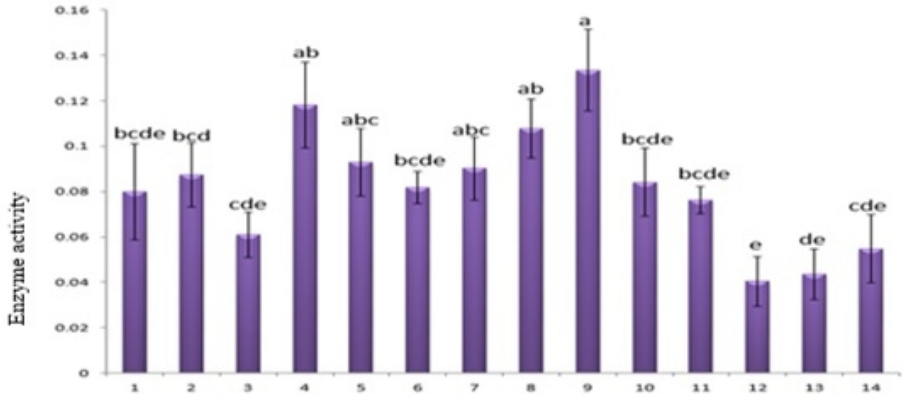

Fig. 6. Densitometric activity mean of POX3 in seedling of 14 wheat genotypes.

Table 3: Mean activity of POX2 and POX3 isozymes of wheat seedlings in four levels of NaCl salinity.

Salinity (mM)	POX2	POX3
0	0.0828 bc	0.0738 ab
100	0.0801 c	0.0703 b
200	0.1136 a	0.0959 a
300	0.1069 ab	0.0889 ab

Different letters in each column explain significant difference

It is worthy to mention that peroxidase enzymes change their activity scheme under stress condition. In this regard, under stress, oxidative development is the result of lacking balance between ROS formation and their ability of detoxification (Cechin *et al.*, 2010). In peroxidase system of advanced plants, there are multiple isoforms which are regulated in an advanced fashion and react to different exogenous factors (Jang *et al.*, 2004).

Table 3 indicates that levels of salinity had a significant effect on the average activity of POX2 and POX3 enzymes. For these isozymes, the highest activity mean is recorded at the levels of 200 mM and 300 mM salt. Other researchers confirmed the significant increase of POX enzyme under salt stress (induced by 0.4 M NaCl) for 48 hours after applying the stress (Nagesh Babo & Davaraj, 2008; Souza & Davaraj, 2010). It seems that with the addition of stress in the environment, in order to maintain the growth and tolerate the unfavorable condition, plant tries to respond by producing and increasing significantly the level of POX. Outcome results of this study shade light on this fact that among evaluated genotypes, which were studied under tension and different levels of stress, there were no specific increasing or decreasing trend in the level of different enzymes isozymes, which it could be due to different environmental and genetic factors and it also might be resulted from interaction of enzymatic effects at the different levels of stress. Moreover, there was no significant difference between degrees of drought tolerance in phenotypes with these isozymes activity level.

Because peroxidase enzyme, which exists in both cytosol and chloroplast, can effectively discard H_2O_2 which is produced in wheat under oxidative stresses (Zhang *et al.*, 2004), so relative increase in activity of this enzyme under stress is probably a promising indicator of accumulation of H_2O_2 under salt stress, which is to be remedied by plant via increasing producing POX. Sharifi *et al.*, 2012, also reported that in wheat lineages under stress condition, peroxidase activity will increase.

Variance analysis of data proved that studied genotypes there is a significant difference (P<1%) between SOD1 and SOD2 isozymes activity, but levels of salinity (Table 2) and also interaction of genotype x salinity were not significant. This reflects an equal reaction of evaluated genotypes in terms of SOD enzyme activity in exerted salt levels.

Munts and Tester (2008) maintain that during oxidative stress, different genotypes of plants show different antioxidant capacity to combat its following damages. In this experiment, studied genotypes were showing disparate and significant SOD enzyme activity in relation with each other from which it could be an evidence on existing diversity of SOD enzyme activity among studied cases.

Mean comparison of quantitative SOD1 isozyme activity showed that the lowest mean for activity of this isozyme is referred to resulted 6, 12, and 15 genotypes (Fig. 7) and as for SOD2 isozyme the least activity for 4, 8, and 11 genotypes were observed; the highest enzyme activity mean was recorded for 13, 9, 6, and 1 genotypes which bear no significant difference with 2, 7, and 10 types (Fig. 8). Hinged on this, it could be seen that POX isozymes activity doesn't have a considerable correlation with the degree of drought tolerance of wheat cultivars and SOD isozymes activity.

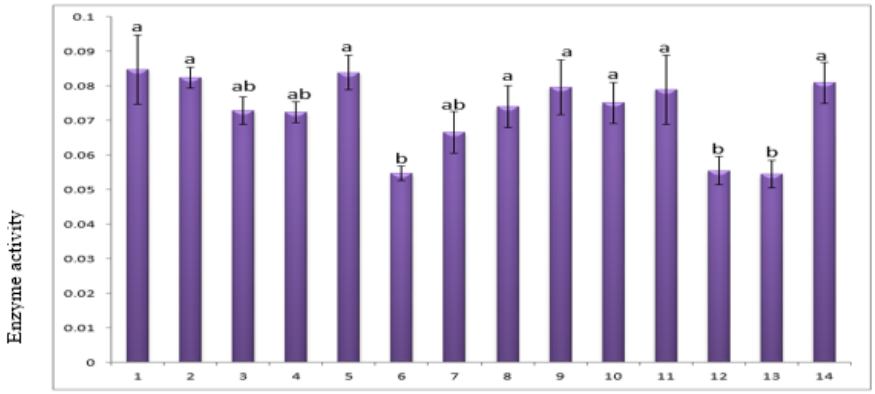

Fig. 7. Densitometric activity of SOD1 enzyme in wheat genotype seedlings.

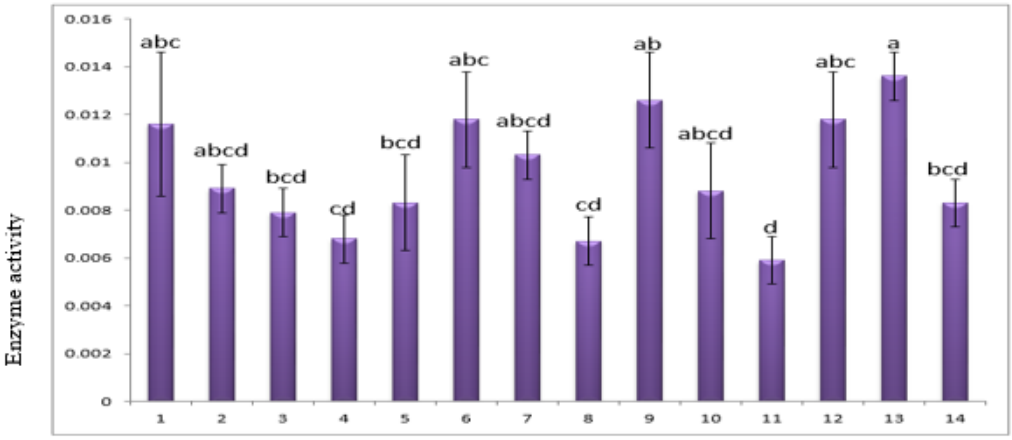

Fig. 8. Densitometric activity of SOD2 enzyme in wheat genotype seedlings.

Based upon the other studies, superoxide dismutase (SOD) is a strong antioxidant and obliterates the first compound which is produced from one-capacity reduction of oxygen, i.e. radical superoxide, so SOD is deemed the primary defense wall against reactive oxygen species (Crouise & Docaraliv, 2008). Furthermore, different investigations showed that in response to different environmental and abiotic stresses including high light intensity (Mittler, 2002), salt (Sairam *et al.*, 2002), and drought (Badavi *et al.*, 2003), SOD enzyme activity in cell will augment. Carried out experiments by Sairam *et al* (1998) on wheat and those done by Manivannan *et al* (2008) on sunflower revealed that the activity of this enzyme under drought stress is much more than that in normal condition.

In this study a significant correlation between POX2 isozyme and other isozymes was not observed, while the maximum positive and significant (P<1%) correlation coefficient was resulted from POX1 and POX3 and this was also significant (P<5%) for POX1 and SOD1 and CAT and SOD2 (Fig. 8).

REFERENCES

Askari, H., Edqvist, J., Hajheidari, M., Kafi, M. & Salekdeh, G. H. (2006). Effects of salinity levels on proteome of Suaeda aegyptiaca leaves. *Proteomics*. **6**: 2542- 2554.

Aydin, A., Kant, C. & Turan, M. (2011). Hydrogel substrate alleviates salt stress with increase antioxidant enzymes activity of bean (*Phaseolus vulgaris* L.) under salinity stress. *African Journal of Agricultural Research*. **6**: 715-724.

Badawi, G. H., Yamauchi, Y., Shimada, E., Sasaki, R., Kawano, N., Tanaka, Ku. & Tanaka, K. (2003). Enhanced tolerance to salt stress and water deficit by overexpressing superoxide dismutase in tobacco (*Nicotiana tabacum*) chloroplasts. *Plant Science*. **166**: 919- 928.

Cechin, I., Corninanil, N., Fumisl, T. F. & Catanco, C. (2010). Differential responses between mature young leaves of sunflower plants to oxidative stress casused by water deficit. *Ciencia Rural, Santa Maria.* **40**: 1290-1294.

Dirk, I. & Montago, M. V. (2003). Oxidative Stress in Plants. CRC Press.

Luna, C. M, Pastpri, G. M., Driscoll, S., Groten, K., Bernard, S. & Foyer, C. H. (2004). Drought controls on H_2O_2 accumulation, catalase (CAT) activity and CAT gene expression in wheat. *Journal of Experimental Botany*. **56**: 417- 423.

Manivannan, P., Jaleel, C. A., Somasundaram, R. & Panneerselvam, R. (2008). Osmoregulation and antioxidant metabolism in drought-stressed *Helianthus annuus* under triadimefon drenching. *Comptes Rendus Biologies*. **331**: 418- 425.

Noreen, Z. & Ashraf, M. (2009). Assessment of variation in antioxidative defense system in salt-treated pea (*Pisum sativum*) cultivars and its putative use as salinity tolerance markers. *Journal of Plant Physiology*. **166**: 1764- 1774.

Mittler, R., (2006). Oxidative stress, antioxidant and stress tolerance. Trends in plant science. **7**: 405- 410.

Mittler, R. & Zilinskas, B. A. (1994). Reulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought. *Plant Journal*. **5**: 397- 405.

Munns, R. & Tester, M. (2008). Mechanisms of salinity tolerance. *Plant Biology*. **59**: 651- 681.

Nagesh Babu, R. & Devaraj, V. R. (2008). High temperature and salt stress response in French bean (Phaseolus vulgaris). *Australian Journal of Crop Science*. **2:** 40- 48.

Olson, P. D. & Varner, J. E. (1993). Hydrogen Peroxides and Lignification. *Plant Journal***. 4**: 887- 892.

Sairam, R. K. & Srivastava, G. C. (2002). Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. *Plant Science*. **162**: 897- 904.

Sairam, R. K., Deshmukh, P. S. & Sexena, D. C. (1998). Role of antioxidant systems in wheat genotypes tolerance to water stress. *Biologia Plantarum.* **41**: 387- 394.

Sairam, R. K. & Srivastava, G. C. (2001). Water stress tolerance of wheat *Triticum aestivum* L.: Variation in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotype. *Journal of Agronomy and Crop Science.* **186**: 63- 70.

Sharifi, P., Amirnia, R., Majidi, E., Hadi, H., roustaii, M., Nakhoda, B., Alipoor, H. M. & Moradi, F. (2012). Relationship between drought stress and some antioxidant enzymes with cell membrane and chlorophyll stability in wheat lines. *African Journal of Microbiology Research*. **6**: 617- 623.

Singh, A., Saini, M. L. & Behl, R. K. (2004). Seed germination and seedling growth of citrus (*Cytrus* species) root stocks under different salinity regimes. *Indian Journal of Agriculture Science.* **74**: 246- 248.

Soltis, D. E. & Soltis, P. S. (1990). Isozymes in plant Biology. Chapman and Hall, London. pp: 259.

Souza, M .R. D. & Devaraj, V. R. (2010). Biochemical responses of Hyacinth bean (*Lablab purpureus*) to salinity stress. *Acta Physiologiae Plantarum*. **32**: 341- 353.

Tan, Y., Liang, Z. S., Hongbo, H. B. & Du, F. (2006). Effect of water deficits on the activity of anti-oxidative enzymes and osmoregulation among three different genotypes of Radix Astragali at graining stage. Colloids and Surfaces. B: *Biointerfaces*, **49**: 59- 64

Turkan, I., Bor, M., Ozdemir, F. & Koca, H. (2005). Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. *Plant Science*. **168**: 223- 231.

Valizadeh, M., Mohayeji, M., Yasinzadeh, N., Nasrullazadeh, S. & Moghaddam, M. (2011). Genetic diversity of synthetic alfalfa generations and cultivars using tetrasomic inheritd allozyme markers. *Journal of Agricultural Science and Technology*. **13**: 425- 430.

Zhang, F., Guo, J. K., Yang, Y. I. & Zhang, L. X. (2004). Changes in the pattern of antioxidant enzymes in wheat exposed to water deficit and rewatering. *Acta Physiologiae Plantarum.* **26**: 345- 352.