

Research Article

Role of Abscisic Acid and Water Stress on the Activities of Antioxidant Enzymes in Wheat

Hadeesa Naz and Nadia Khan

Department of Genetics, University of Karachi, Karachi 75270, Pakistan

Abstract: Eight wheat varieties (Chinese Spring, Pavon, Gabo, Saleem-2000, Zamindar-04, Siren, NR-264 and Marvi) were compared for their response to exogenous application of abscisic acid (ABA), Water Stress (WS) and Control (C) during *invitro* condition. Their responses were studied in the form of seedlings growth and antioxidant enzymes. Exogenous application of ABA reflected ameliorating effect on catalase activity. Water stress treatment led to increase in levels of catalase except Pavon. Increased activity of antioxidant enzymes showed tolerance capacity under water stress. Correlation coefficient analysis reflected negative and significant relationship between total protein contents and peroxidase and catalase. Further, investigations are needed to enhance the understanding on the effect of different abiotic stresses and growth hormones during early seed development.

Keywords: Abscisic acid, catalase, correlation, peroxidase, water stress and wheat

INTRODUCTION

Drought stress is among the most damaging abiotic stresses that affect agriculture. Due to current climate change scenario, plants suffer from erratic water stress (Zhang *et al.*, 2006). Wheat is the main cereal crop of Pakistan and it is a prime food of the people. It contributes 14.4% to the value added in agriculture and 3.1% to GDP (Anonymous, 2010). Plant response to water stress include morphological and biochemical changes and later as water stress become more severe to functional damage and loss of plant parts (Sangtarash, 2010). Phytohormones are considered as main signals during stress conditions (Davies, 1995; Pospisilova *et al.*, 2005). And almost all processes in the life of a plant are directly or indirectly influenced phytohormones (Pospisilova, 2003). Especially abscisic acid (ABA) has been considered to be one of the main hormone which triggers various acclimations processes under water stress conditions (Zhu, 2002). Under water stress condition exogenously applied ABA stimulated the synthesis of proteins in different species (Riccardi *et al.*, 1998). ABA also expresses the gene encoding enzymes that participate in the repair of spontaneous protein damage (Mudgett and Clark, 1996). Moreover, ABA induced increase in the osmolyte might also help in stabilizing the proteins under water stress (Noiraud *et al.*, 2001). Exogenous application of ABA has also been reported to significantly increase tolerance (Jiang and Zhang, 2004; Farooq and Bano, 2006; Iqbal *et al.*, 2012; Khan, 2013).

Increasing evidence indicates that one mode of ABA action may be related to its role in the oxidative stress in plant cells. It has been documented that ABA can cause an increased generation of O₂ (Jiang and

Zhang, 2001) and H₂O₂ (Guan *et al.*, 2000; Jiang and Zhang, 2001; Zhang *et al.*, 2001). To alleviate adverse effects of reactive oxygen species, plants have evolved an antioxidant defense system that include enzymes like superoxide dismutase, peroxidase and catalase (Agarwal and Pandey, 2004; Bano *et al.*, 2012).

The objective of this study was an effort made to investigate the role of ABA under water stress and also interrelationship between antioxidant enzymes such as peroxidase and catalase in the seedlings of different wheat varieties exposed to water stress and ABA condition.

MATERIALS AND METHODS

Plant materials and stress treatments: A laboratory experiment was conducted in 2011; the experiment was carried out in a factorial on a randomized block design with three replications. Seeds of eight different wheat varieties (Chinese Spring, Pavon, Gabo, Saleem-2000, Zamindar-04, Siren, NR-264, Marvi) were used. Seeds were surface sterilized with 0.1% bleach solution, thoroughly washed three times with distilled water and were placed in petri plates containing sterilized filter paper. In each petri plate eight seeds were placed. Water was added in each petri plate at regular intervals of time. After one week seedlings were exposed to three different treatments i.e., Water Stress (WS), abscisic acid (ABA) and control (C). No water was added at water stress condition whereas 30 μM of ABA was applied under ABA condition and there was regular watering under control condition. After one week, treated and untreated seedlings were harvested and biochemical analysis was done.

Growth parameters: Shoot and root length was measured in cms and then average was calculated. Fresh weight of all seedlings was measured in gm with the help of electronic balance. Seedlings were crushed into pre chilled pestle and mortar containing 2 mL cold homogenizing buffer solutions (pH7.5) per gm fresh weight of material. The homogenate were centrifuge first at 4,000 rpm for 10 min and then 10,000 rpm for 15 min. The resultant supernatant was collected and used for biochemical analysis.

Biochemical analysis: The protein content of determined follows the method of Lowry *et al.* (1951). Quantitative estimation of peroxidase was done as described by Everse *et al.* (1994), the assay mixture containing 10µl plant extract, 3.75 mL of 0.15 M phosphate buffer (pH5.6), 100 µL of 176 mM H₂O₂ and the same amount of 0.1 M guaiacol as H₂O₂ was left for 8 min and then the reaction mixture was shaken well after adjusting the total volume up to 10 mL with distilled water. The increase in absorbance due to guaiacol oxidation was recorded at 470 nm. Catalase was assayed by monitoring the decrease of absorbance. The reaction mixture consisted of 50 µL of enzyme, 2.5 mL of 50 mM potassium phosphate buffer (PH 7.6), 200 µL of 12.5 mM H₂O₂ and 250 µL of distilled water. The absorbance of the mixture was recorded at 240nm. This method was described by Aebi (1984).

Statistical analysis: Analysis of variance, mean comparisons and correlation coefficient analysis were performed through computer program SPSS software version 11.0.

RESULTS AND DISCUSSION

Growth parameters: The mean root length of the wheat genotypes responded differently to treatments (Table 1). Zamindar-04 showed maximum root length (7.68 cm) whereas Marvi had a minimum root length (2.8 cm) under control condition. All genotypes showed reduction in root length except Marvi at ABA condition (Table 2). In this study the root length of wheat genotypes has affected by water stress. The less retardation of growth was found in Marvi among eight genotypes of wheat. Imposition of water deficit stress led to inhibition of growth in wheat seedlings. Maintenance of root growth during water deficit is beneficial to maintain adequate plant water supply (Pinheiro *et al.*, 2004). However Gabo showed higher reduction under water stress. Baloch *et al.* (2012) reported that root length had a high percentage decrease due to osmotic stress. Marvi showed a consistent performance under ABA condition with respect of root length. The continued growth of roots in water stressed soil is particularly important to avoid the effect of water stress (Dhanda *et al.*, 1995; Misra, 1990, 1994). In

Table 1: Mean squares for root length of wheat varieties among different treatments

Sources of variation	df	Root length (cms)
Replications	2	0.714
Treatments (T)	2	78.319**
Varieties (V)	7	17.582**
V×T	14	7.70
Error	32	4.806

*, **: Significant at 0.05 and 0.01 level of probability, respectively

Table 2: Mean values of wheat varieties for root length among different treatments

Varieties	Control	WS	ABA
Chinese spring	6.30±0.30 ^a	4.80±0.47 ^a	4.20±0.33 ^{bc}
Gabo	6.73±0.33 ^a	3.15±0.30 ^b	2.69±0.26 ^d
Marvi	2.80±0.76 ^c	2.60±0.52 ^b	6.06±0.40 ^a
NR-264	6.67±0.56 ^a	3.18±0.30 ^b	3.90±0.51 ^{bc}
Pavon	4.35±0.62 ^b	2.50±0.36 ^b	2.30±0.51 ^d
Saleem-2000	6.49±0.30 ^a	3.10±0.32 ^b	3.27±0.34 ^{cd}
Siren	7.66±0.31 ^a	4.59±0.30 ^a	3.37±0.25 ^{cd}
Zamindar-04	7.68±0.29 ^a	5.47±0.24 ^a	4.71±0.25 ^b

Means followed by the same letter within columns are non-significantly different ($p \leq 0.05$) according to DMR test

general, root length of the drought sensitive genotypes was significantly reduced as compared to drought tolerant genotypes (Devi *et al.*, 2012). The development of root system in water deficit conditions appears to be a very viable criterion to select for water stress because it takes the moisture from lower layers of soil and also obviously suffers from more than any other seedling trait (Baloch *et al.*, 2012). Exogenous supplied ABA under water stress contributes to protective role/reactions aimed to decrease its injurious effect on growth and acceleration of growth resumption (Sakhabutdinova *et al.*, 2003).

Total protein contents: Table 3 revealed non-significant differences among treatments whereas genotypes showed significant differences. Contrasting reports regarding the changes in protein contents are available in literature (Iqbal *et al.*, 2010). Increasing water stress was found to cause a significant reduction in soluble protein content in moth beans (Garg *et al.*, 2001) and *vigna radiata* L. (Farooq and Bano, 2006). The increase in total soluble proteins under water stress in some accessions and decrease in others is consistent with previous findings (Bensen *et al.*, 1988; Riccardi *et al.*, 1998; Ti-Da *et al.*, 2006) water stress increased some soluble proteins and decreased others.

Peroxidase: The specific activity of peroxidase enzyme non-significantly differed under different treatments and genotypes (Table 3). Oxidative damages in plant caused by biotic and abiotic stresses are usually reduced by enzymatic antioxidants (superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase and by non-enzymatic antioxidants (Ismail *et al.*, 2005). The ability of antioxidant enzymes to scavenge ROS and reduce their damaging effects may confer drought resistance to plants. Yang *et al.* (2009) exhibited that as compared with 100% field capacity, at 25% field

Table 3: Mean squares for total protein content, POX and CAT among different treatments

Sources of variation	df	Total protein content	POX	CAT
Replications	2	25681.158	6.978	0.248
Treatments (T)	2	71929.118	5.616	24.631**
Varieties (V)	7	109290.878**	117.085	11.937**
V×T	14	15530.507	5.771**	7.503**
Error	32	27090.788	4.355	3.743

Table 4: Mean values of wheat varieties for CAT activity among different treatments

Varieties	Control	WS	ABA
Chinese Spring	0.95±0.09 ^b	1.58±0.02 ^b	0.87±0.08 ^c
Gabo	1.53±0.12 ^b	2.96±0.07 ^b	1.78±0.26 ^{bc}
Marvi	1.56±0.07 ^b	2.78±0.20 ^b	2.07±0.19 ^b
NR-264	1.36±0.08 ^b	2.83±0.18 ^b	1.74±0.04 ^{bc}
Pavon	4.10±0.08 ^a	3.65±0.79 ^{ab}	4.08±1.04 ^a
Saleem-2000	1.33±0.34 ^b	1.51±0.38 ^b	1.88±0.31 ^{bc}
Siren	1.50±0.59 ^b	1.60±0.22 ^b	0.89±0.15 ^c
Zamindar-04	1.36±0.36 ^b	7.53±2.04 ^a	2.06±0.38 ^b

Table 5: Correlation coefficient between total protein contents and peroxidase and catalase under different treatments

Treatments	TP	POX	CAT
C	TP	-0.590**	-0.462**
	POX		0.544**
WS	TP	-0.495**	-0.544**
	POX		0.561**
ABA	TP	-0.528**	-0.341*
	POX		0.639**

** : Significant at 5% probability

capacity the increased activities of CAT, SOD, POD in poplar species.

Catalase: The catalase activity of the treatments and genotypes differed significantly (Table 4). In the control condition, Pavon had a higher activity of catalase i.e., 4.10 U.mg⁻¹ protein/min and Chinese Spring had a lesser catalase activity (0.95 U.mg⁻¹ protein/min). All genotypes reflected enhanced catalase activity except Pavon that withstand their activity where no supplied of water. Exogenously ABA induced to wheat seedlings that most of the genotypes reflected increased activity but few had decreased than control. Yong *et al.* (2006) found that the rules of SOD, CAT and POX activity change are similar, which indicated that these three enzymes cooperated with each other during water deficits. However, reports on the effect of stresses on CAT activities vary (Salekjalali *et al.*, 2012). Increased, decreased, or unchanged CAT activities under drought stress have been observed by Jiang and Huang (2001), Sobkowiak *et al.* (2004) and Salekjalali *et al.* (2012). Higher levels of antioxidant enzymes are related to drought tolerance in different plants (Khanna-Chopra and Selote, 2007; Hameed *et al.*, 2011; Bhagi *et al.*, 2013).

Drought stress caused an increase in catalase activity in leaf tissues of both wheat cultivars (Valifard *et al.*, 2012). Catalase plays a key role in detoxification of H₂O₂ produced specially in chloroplasts under oxidative stresses (Blokhina *et al.*, 2003). Our results agree with those reported by Ismail *et al.* (2005) who found higher catalase activity in drought tolerant bean

plants as compared to drought sensitive. Similar results were reported by Valifard *et al.* (2012), Devi *et al.* (2012) and Khan (2013) in wheat cultivars. Increase in CAT activity has been shown to be related with increase in stress tolerance capacity (Kraus *et al.*, 1995; Devi *et al.*, 2012).

Correlation coefficient: Table 5 depicted correlation coefficient among different treatments and genotypes. Total protein content showed negative and significant correlation with peroxidase and catalase under control condition. Similar results were obtained under rest of the stresses. These results indicated that increase in protein content caused decrease in antioxidant enzymes. Peroxidase exhibited positive and significant correlation with catalase at irrigated, water stress and ABA condition. Positive correlation occurs between endogenous ABA and antioxidant activities of SOD and POD were reported by Bano *et al.* (2012).

CONCLUSION

Activities of antioxidant enzymes could be a useful tool for depicting drought tolerance of wheat, which could be useful to plant breeders for developing drought-tolerant cultivars. It could be suggested that ABA played a positive role for plant to improve its antioxidant defense mechanism in plants. Further studies are needed to confirm the role of antioxidant enzymes for understanding drought tolerance in a large number of genotypes.

ACKNOWLEDGMENT

The authors gratefully acknowledge Dr. Iftikhar Hussain Khalil, Cereal Crop Research Institute (CCRI), Pirsabak, Nowshera, KPK, Pakistan; Dr. Larik, Sindh Agriculture University, Tandojam, Pakistan; Dr. Ali Akbar Shahnejat Bushehri, University of Tehran, Karaj, Iran and Dr. J. Pena, CIMMYT, Mexico that provided germplasm to conduct research. This research study was financially supported by the Deans Science Research Grant for the year 2011-2012.

ABBREVIATIONS

Abcisic Acid (ABA)
Water Stress (WS)
Control (C)
Catalase (CAT)
Peroxidase (POX)

REFERENCES

- Aebi, H., 1984. Catalase *in vitro*. Method Enzymol., 105: 121-126.
- Agarwal, S. and V. Pandey, 2004. Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. Biol. Plantarum, 48: 555-560.
- Anonymous, 2010. Pakistan Economic Survey 2009-2010. Finance Division, Economic Advisory Wing, Government of Pakistan, Islamabad.
- Baloch, M.J., D. Jim, A.K. Abdul, D. Mike, A.J. Wajid and A.C. Siraj, 2012. Assessment of wheat cultivars for drought tolerance via osmotic stress imposed at early seedling growth stages. J. Agric. Res., 50: 299-310.
- Bano, A., F. Ullah and A. Nosheen, 2012. Role of abscisic acid and drought stress on the activities of antioxidant enzymes in wheat. Plant Soil Environ., 58: 181-185.
- Bensen, R., J.S. Boyer and J.E. Mullet, 1988. Water deficit-induced changes in abscisic acid, growth, polyamines, translatable RNA in soybean hypocotyls. Plant Physiol., 88: 289-294.
- Bhagi, P., K.Z. Vikramjit and A.K. Gupta, 2013. Antioxidant response and Lea genes expression under salt stress and combined salt plus water stress in two wheat cultivars contrasting in drought tolerance. Ind. J. Exp. Biol., 51: 746-757.
- Blokhina, O., E. Virolainen and K.V. Fagerstedt, 2003. Antioxidants, oxidative damage and oxygen deprivation stress: A review. Ann. Bot., 91: 179-194.
- Davies, P.J., 1995. Introduction. In: Davies, P.J. (Ed.), Plant Hormones, Physiology, Biochemistry and Molecular Biology. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp: 1-12.
- Devi, R., K. Narinder and K.G. Anil, 2012. Potential of antioxidant enzymes in depicting drought tolerance of wheat (*Triticum aestivum* L.). Indian J. Biochem. Biophys., 49: 257-265.
- Dhanda, S.S., R.K. Behl and L. Elbassam, 1995. Breeding wheat genotypes for water deficit environments. Landbauforsch. Volk., 45: 159-167.
- Everse, J., M.C. Johnson and M.A. Marini, 1994. Peroxidative Activities of Hemoglobin and Hemoglobin Derivatives. In: Everse, J., K.D. Vandegriff and R.M. Winslow (Eds.), Methods in Enzymology. Academic Press, San Diego, 231: 547-561.
- Farooq, U. and A. Bano, 2006. Effect of Abscisic acid and chlorocholine chloride on nodulation and biochemical content of *vigna radiata* L. under water stress. Pak. J. Bot., 38: 1511-1518.
- Garg, B.K., S. Kathju and U. Burman, 2001. Influence of water stress on water relations, photosynthetic parameters and nitrogen metabolism of moth bean genotypes. Biol. Plantarum, 44: 289-292.
- Guan, L., J. Zhao and J.G. Scandalios, 2000. Cis-elements and trans-factors that regulate expression of maize Cat1 antioxidant gene in response to ABA and osmotic stress: H₂O₂ is the likely intermediary signaling molecule for the response. Plant J., 22: 87-95.
- Hameed, A., N. Bibi, J. Akhter and N. Iqbal, 2011. Differential changes in antioxidants, proteases and lipid peroxidation in flag leaves of wheat genotypes under different levels of water deficit conditions. Plant Physiol. Bioch., 49: 178-185.
- Iqbal, S., B. Asghari and I. Noshin, 2010. Drought and abscisic acid (ABA) induced changes in protein and pigment contents of four wheat (*Triticum aestivum* L.) accessions. J. Agric. Res., 48: 1-13.
- Iqbal, S., B. Asghari and I. Noshin, 2012. Abscisic acid (ABA) seed soaking induced changes in physiology of two wheat cultivars under water stress. Pak. J. Bot., 44: 51-56.
- Ismail, T., B. Melike, O. Filiz and K. Hulusi, 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 Sci., 168: 223-231.
- Jiang, M. and J. Zhang, 2001. Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. J. Plant Cell Physiol., 42: 1265-1273.
- Jiang, Y. and B. Huang, 2001. Drought and heat stress injury to two cool season turf grasses in relation to antioxidant metabolism and lipid peroxidation. Crop Sci., 41: 436-442.
- Jiang, M.Y. and J.H. Zhang, 2004. Abscisic acid and antioxidant defense in plant cells. Acta Bot. Sin., 46: 1-9.
- Khan, N., 2013. Changes in the antioxidant enzymes activity of wheat seedlings under abscisic acid (ABA) and water stress. Int. J. Biol. Biotech., 10: 79-82.
- Khanna-Chopra, R. and D.S. Selote, 2007. Acclimation to drought stress generates oxidative stress tolerance in drought-resistant than susceptible wheat cultivar under field conditions. Environ. Exp. Bot., 60: 276-283.
- Kraus, T.E., B.D. McKersie and R.A. Fletcher, 1995. Paclobutrazol-induced tolerance of wheat leaves to paraquat may involve increased antioxidant enzyme activity. J. Plant Physiol., 145: 570-576.
- Lowry, O.H., N.J. Rosebrough, A.L. Far and R.J. Randal, 1951. Protein measurements with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
- Misra, A.N., 1990. Seedling vigour and prediction of drought resistance in pearl millet genotypes (*Pennisetum americanum* L., Leeke). Beitr. Trop. Landwirt., 28(2): 155-159.

- Misra, A.N., 1994. Pearl millet (*Pennisetum glaucum* L.R.Br.) seedling establishment under variable soil moisture levels. *Acta Physiol. Plant*, 16: 101-103.
- Mudgett, M.B. and S. Clark, 1996. A distinctly regulated protein repair L-isoaspartyl methyltransferase from *Arabidopsis thaliana*. *Plant Mol. Biol.*, 30: 723-737.
- Noiraud, N., L. Maurousset and R. Lemoine, 2001. Identification of a mannitol transporter, AgMaT1, in celery phloem. *Plant Cell*, 13: 695-705.
- Pinheiro, H.A., R.M. Damatta, A.R.M. Chaves, E.P.B. Fontes and M.E. Loureiro, 2004. Drought tolerance in relation to protection against oxidative stress in clones of *Coffea canephora* subjected to long-term drought. *Plant Sci.*, 167: 1307-1314.
- Pospisilova, J., 2003. Participation of phytohormones in the stomatal regulation of gas exchange during water stress. *Biol. Plantarum*, 46: 491-506.
- Pospisilova, J., M. Vagner, J. Malbeck, A. Travnickova and P. Batkova, 2005. Interaction between abscisic acid and cytokinins during water stress and subsequent rehydration. *Biol. Plant*, 49: 533-540.
- Riccardi, F., P. Gazeau, D. de Vienne and M. Zivy, 1998. Protein changes in response to progressive water deficit in maize. *Plant Physiol.*, 117: 1253-1263.
- Sakhabutdinova, A.R., D.R. Fatkhutdinova, M.V. Bezrukova and F.M. Shakirova, 2003. Salicylic acid prevents the damaging action of stress factors on wheat plants. *Bulg. J. Plant Physiol., Special Issue*, pp: 314-319.
- Salekjalali, M., H. Raheem and J. Behboud, 2012. Effects of soil water shortages on the activity of antioxidant enzymes and the contents of chlorophylls and proteins in barley. *American-Eurasian J. Agric. Environ. Sci.*, 12: 57-63.
- Sangtarash, M.H., 2010. Responses of different wheat genotypes to drought stress applied at different growth stages. *Pak. J. Biol. Sci.*, 13: 114-119.
- Sobkowiak, R., K. Rymer, R. Rucinska and J. Deckert, 2004. Cadmium-induced changes in antioxidant enzymes in suspension culture of soybean cells. *Acta Biochim. Pol.*, 51: 219-222.
- Ti-Da, G.E., S.U.I. Fang-Gong and B.A. Ping, 2006. Effects of water stress on the protective enzymes and lipid peroxidation in roots and leaves of summer maize. *Agric. Sci. China*, 5: 291-298.
- Valifard, M., A. Moradshahi and B. Kholdebarin, 2012. Biochemical and physiological responses of two wheat (*Triticum aestivum* L.) cultivars to drought stress applied at seedling stage. *J. Agric. Sci. Technol.*, 14: 1567-1578.
- Yang, F., X. Xu, X. Xiao and C. Li, 2009. Responses to drought stress in two poplar species originating from different altitudes. *Biol. Plant*, 53: 511-516.
- Yong, T., L. Zong-Suo, S. Hong-Bo and D. Feng, 2006. Effect of water deficits on the activity of antioxidative enzymes and osmoregulation among three different genotypes of *Radix astragali* at seedling stage. *Colloid Surface B*, 49: 60-65.
- Zhang, J., W. Jia, J. Yang and A.M. Ismail, 2006. Role of ABA in integrating plant responses to drought and salt stresses. *Field Crop Res.*, 97: 111-119.
- Zhang, X., L. Zhang, F. Dong, J. Gao, D.W. Galbraith and C.P. Song, 2001. Hydrogen peroxide is involved in abscisic acid-induced stomatal closure in *vicia faba*. *Plant Physiol.*, 126: 1438-1448.
- Zhu, J.K., 2002. Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Biol.*, 53: 247-273.