

C2	2.59	2.39
C3	1.91	1.61
C4	1.14	1.90
E1	1.85	1.86
E2	2.43	2.39
E3	1.81	1.91
E4	2.36	2.25

Higher proline content in wheat plants after water stress has been reported by Errabii et al. (2006), Patel and Vora (1985) and Vendruscolo et al. (2007). Many reports from crops and other plants have proved this (Wang and Li, 2000; Wang et al., 2003; Errabii et al., 2006; Shao et al., 2006). This increase in free proline content due to water deficit has been reported by many authors (Delauney & Verma, 1993; Johari-Pireivatlou et al., 2010).

Tatar and Gevrek (2008) suggested that proline is mainly involved in protection against oxidative stress that osmotic adjustment during the onset of water stresses. It has been also proven that proline has an essential role in stabilising proteins and cellular membranes in plant cells in the

presence of high levels of osmolytes (Errabii et al., 2006; Farooq et al., 2009). In addition, Vendruscolo et al. (2007) suggested that proline plays an important role in water stress tolerance mechanism(s) in plants due to its ability in opposing oxidative stress; and considered this as the most important strategy in plants to overcome water deficit effects.

3.4 Amylase Activity

Amylase shows increased activity in experimental plant as compare to control. In Aestivum wheat the higher values of free β -amylase was observed in NI 5439 variety and MACS 2496 variety (945 and 1014 units/gm) resp. The highest bound β -amylase value was observed in MACS 6222 and MACS 2496 (198.5 and 185.7 units/gm) resp as shown in table 4.

These results are consistent with other studies reporting the increased amylase activity

Table 4: Mean of different enzyme activities in Aestivum wheat

S. No.	Aestivum Wheat (2012-2013)				Aestivum Wheat (2014-15)			
	Free β -Amylase (units/gm)	Bound β -Amylase (units/gm)	Peroxidase (units/gm)	Catalase (units/gm)	Free β -Amylase (units/gm)	Bound β -Amylase (units/gm)	Peroxidase (units/gm)	Catalase (units/gm)
C1	728.60	158.57	2.5	2.030	928.57	158.57	1.7	2.41
C2	814.30	128.57	2.8	1.488	814.28	128.55	2.4	2.73
C3	857.14	135.71	2.2	1.353	960.00	125.54	1.9	2.70
C4	871.40	155.71	2.1	1.015	871.42	150.71	2.3	2.35
E1	814.30	191.43	2.9	3.654	1014.28	185.71	3.2	3.52
E2	900.00	198.57	3.2	3.383	900.00	157.14	3.8	3.76
E3	971.40	162.86	3.4	3.586	961.42	160.85	3.6	3.44
E4	945.00	187.14	3.5	4.263	981.20	167.14	3.7	3.47

in response to drought stress in wheat (Bakalova et al., 2004 & Csiszar et al., 2005)

Catalase enzyme activity (CAT):

Enhanced catalase enzyme activity was observed in Aestivum wheat. The highest catalase enzyme activity was observed in NI 5439 and MACS 6222 variety (4.26 and 3.76 units/gm resp.) for both the years as shown in table no.5.

During drought stress in wheat (*Triticum aestivum L.*) activity of enzymatic antioxidant CAT increased to manage the oxidative stress (Mohammad, R. A. and M., Mahdiyeh; 2013) which is similar result found in present investigation.

Catalase (CAT) reacts with H₂O₂ directly to form water and oxygen (Smirnoff 1993, Winston 1990). The decrease in CAT activity could indicate its inactivation by the accumulated hydrogen peroxide induced by water shortage and could be explained partly by photo inactivation of the enzyme. Under irradiation, inactivation of CAT occurs permanently and is mediated through light absorption by the enzyme-bound heme group (Feierabend and Kemmerich 1983, Feierabend and Engel 1986). When plants are not exposed to water stress, resynthesis of CAT compensates for the loss of total activity caused by irradiance.

Catalase is an oxidoreductase, located in peroxysomes and considered as an important enzyme to counter hydrogen peroxide in stress condition, so that at drought condition new isomorphs of it are released and rate of former isomorphs increases (Srivalliet al., 2003; Khanachorpa and Selote, 2007).

In this organelle, H₂O₂ is produced from β -oxidation of fatty acids and photorespiration (Morita et al., 1994). Higher activity of CAT and APX decrease H₂O₂ level in cell and increase the stability of membranes and CO₂ fixation because several enzymes of the Calvin cycle within chloroplasts are extremely sensitive to H₂O₂. A high level of H₂O₂ directly inhibits CO₂ fixation (Yamazaki et al., 2003).

Catalase is responsible for decomposition and detoxification of H₂O₂ in the peroxisomes. The activity of this enzyme is sensitive to heat as well as drought stress (Jiang and Hoang, 2001). Decrease in activity of this enzyme may relate to either photo inactivation of the enzyme (Pollen, 1997) which is a sign for advent of light stress in the plant the usually cause photo-inhibition of photosystem II, and this condition itself leads to H₂O₂ concentration and damage to cell membrane (Jang, 2004) or prevention of new enzyme synthesis that occur in darkness, is another factor which decreases the activity of this enzyme (Datet al., 1998).

3.5 Peroxidase

In the present study, during water stress all wheat cultivars showed increased activity of peroxidase enzyme as shown in (Table No. 4). Similar trend was observed for Aestivum wheat under water stress condition. In Aestivum wheat MACS 6222 and NI 5439 showed highest peroxidase enzyme activity as compared to control as shown in table no.5. An increase of POD activity was observed in other studies under drought (Badiani *et al.* 1990; Dwivedi *et al.* 1979) and other stress conditions such as salt (Siegel 1993). Under drought hexaploid wheats had higher POD activity which was reported by (Zang and Khirkham, 1994)

4. Conclusion

Present work is initiative for studying the different mechanisms under drought stress conditions. At the global level climatic changes are took place so ultimately water shortage also increased hence there is continuous decrease in wheat production. Acclimation of plants to drought stress is considered to promote antioxidant activity. Antioxidant enzymes like catalase, peroxidase and amylase are related with water deficiency and are considered as main component of antioxidant machinery for drought resistant in plants.

In the present study MACS 2496 variety shows highest carbohydrate content in both control as well as experimental condition for both year. Higher proline accumulation was observed in MACS 2496 variety and it also showed low protein content. This may be due to protein degradation during drought stress and this degradation might be leads to increase in proline content. During water stress condition proline and carbohydrates are act as osmoregulants.

In contrast to this MACS6222 variety showed higher protein content and lower accumulation of carbohydrate and proline in both control as well as experimental plants during both year. As compared to control, experimental plants showed increased value of carbohydrate and proline. MACS6222 variety showed higher peroxidase activity than other three varieties. For acclimatization to drought stress plant shows higher activity of peroxidase. Higher activity of peroxidase in MACS6222 is may be due to lower concentration of osmoregulants and for acclimatization.

Free β -amylase content was more in MACS 2496 in both controls as well as in experimental condition but higher content of bound β -amylase is found in MACS 6145 but it was lowest in NI 5439 Variety. Although NI 5439 Variety had low β - bound amylase activity but it showed higher catalase activity with compare to MACS 2496, MACS 6145 and MACS6222 varieties. MACS 6145 variety showed optimal activity for all these parameter in both conditions for both the year.

From the results obtained during this study we can say that the four different varieties showed systematic increase in enzyme activity particularly peroxidase and catalase under drought stress condition. Out of these four varieties NI 5439 and MACS 6145 showed optimal activity for all the parameters in our study. From this we can conclude that

these two varieties can show the ability of wheat plants to acclimate under drought stress condition.

References

- [1] Bradford, M. M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dibinding. *Anal. Biochem.* **199**: 91.
- [2] Cadenas, S. E., 1989. Biochemistry of oxygen toxicity. *Ann. Rev. Biochem.* **59**: 79-110.
- [3] Davies, K. J. A., 1987. Protein damage and degradation by oxygen radicals. I. General aspects. *J. Biol. Chem.* **262**:9895- 9901.
- [4] Imlay, J. A. and S., Linn, 1988. DNA damage and oxygen radical toxicity. *Science* **240**:1302-1309.
- [5] Egneust, H.; Heber, U. and M., Kirk, 1975 .Reduction of oxygen by the electron transport chain of chloroplasts during assimilation of carbon dioxide. *Biochim. Biophys. Acta.* **408**: 252-268.
- [6] Elstner, E. F., 1987. Metabolism of activated oxygen species. In D. D. Davies (ed.) *The Biochemistry of Plants, Biochemistry of Metabolism*, Academic Press, San Diego, USA. **11**: 253-315.
- [7] Fridovich, I., 1986. Biological effects of superoxide radical. *Arch. Biochem. Biophys.* **247**: 1-11.
- [8] Jang, S., 2004. Variation in antioxidant metabolism of young and mature leaves of *Arabidopsis thaliana* subjected to drought. *Plant sci.* **166**: 459-466.
- [9] Jones, M. M.; N. C., Turner and C.B., Osmond, 1981. Mechanisms of drought resistance. In: L.G. Paleg, D. Aspinall, eds. *Physiology and Biochemistry of Drought Resistance in Plants*. Sydney: Academic Press:15-37.
- [10] 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.
- [11] Kruger, J. E., 1972. Organic and bioorganic chemistry of carbon dioxide. *Cereal Chem.* **49**: 379.
- [12] Liebler, D. C.; Kling, D. S. and D. J., Reed, 1986. Antioxidant protection of phospholipid bilayers by α -tocopherol, Control of α -tocopherol status and lipid per! oxidation by ascorbic acid and glutathione. *J. Biol. Chem.* **150**: 12114-12119.
- [13] Malik, C. P. and M. B., Singh, 1980. In: *Plant Enzymology and Histoenzymology*. Kalayani publisher, New Delhi.
- [14] Peter Bernfield, 1955. In: *Methods of Enzymology* Academic Press, New York, 1: 149.
- [15] Putter, J., 1974. In: *Methods in Enzymatic Analysis*, 2 (Ed. Bergemeyer), Acadmic Press, New York: 685.
- [16] Rabe; E., 1990. Stress physiology: The functional significance of the accumulation of nitrogen-containing compounds. *J Hort Sci* **65**:231.
- [17] Smirnoff, N., 1993. The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytol.* **125**: 27-58.
- [18] Srivalli, B. G.; Sharma and R., Khanna-Chopra, 2003. Antioxidative defence system in an upload rice cultivar subject to increase intensity of water stress following by recovery. *Physiol. Planta* **119**: 503-512.

- [19] Winston, G.W., 1990. Physiochemical basis for free radical formation in cells: production and defenses. *In Stress Responses in Plants: Adaptation and Acclimation Mechanisms*. Edited by Alscher, R.G. and Cumming, J.R. pp. 57-86. Wiley-Liss, Inc., New York.
- [20] Hedge, J. E. and B. T., Hofreiter, 1962. In: Carbohydrate chemistry, 17, Academic Press, New York.
- [21] Hanson, A. D. and W. D., Hitz, 1982. Metabolic responses of mesophytes to plant water deficits. *Ann. Rev. Plant Physiol.* **33**: 163-203.
- [22] Errabi, T.; Gandonou, C. B.; Essalmani, H.; Abrini, J.; Idaomar, M. and N., Skali-Senhaji, 2006. Growth, Proline and ion accumulation in Sugarcane callus cultures under drought-induced osmotic stress and its subsequent relief. *Afr. J. Biotechnol.* **5**(6): 1488-1493.
- [23] Badiani, M., De Biasi, M.G., Colagnola, M. and F., Artemi, 1990. Catalase, peroxidase and superoxide dismutase activities in seedlings submitted to increasing water deficit. *Agrochimica* **34**: 90-102.
- [24] Chaves, M.M.; Pereira, J.S.; Maroco, J.; Rodrigues, M.L.; Ricardo, C.P.P.; Osorio, M.L.; Carvalho, I.; Faria, T. and C., Pinheiro, 2002. How plants cope with water stress in the field. Photosynthesis and growth. *Ann. Bot.* **89**: 907-916.
- [25] Delauney, A. J. and D.P.S., Verma, 1993. Proline biosynthesis and osmoregulation in plants. *Plant J.* **4**: 215-223
- [26] Dwivedi, S., Kar, M. and D., Mishra, 1979. Biochemical changes in excised leaves of *Oryza sativa* subjected to water stress. *Physiol. Plant.* **45**: 35-40.
- [27] Jiang, Y. and N., Huang, 2001. Drought and heat stress injury to two cool season turf grasses in relation to antioxidant metabolism and lipid peroxidation. *Crop sci.* **41**: 436-422.
- [28] Johari-Pireivatlou, M.; Qasimov, N. and H., Maralian, 2010. Effect of soil water stress on yield and proline content of four wheat lines. *African J. Biotechnol.* **9**: 036-040
- [29] Martin, M.; Michell F.; Morgan, J. A.; Scalet, M. and G., Zebri. 1993. Synthesis of osmotically active substances in winter wheat leaves as related to drought resistance of different genotypes. *J. of Agronomy and Crop Science.* **171**: 176-184.
- [30] Mohammadkhani, N. and R., Heidari, 2007. Effects of water stress on respiration, photosynthetic pigments and water content in two maize cultivars. *Pakistan J. Biol. Sci.* **10**: 4022-4028
- [31] Mohammadkhani, N. and R., Heidari, 2008. Drought-induced accumulation of soluble sugars and proline in two maize varieties. *World Appl. Sci. J.* **13**: 448-453.
- [32] Mohammad, R. A. and M., Mahdiyeh, 2013. Antioxidative and biochemical responses of wheat to drought stress, *ARP Journal of Agricultural and Biological Science*, **8**: 1990-6145
- [33] Patel, J. A. and A. B., Vora, 1985. Free proline accumulation in drought stressed plants. *Plant and Soil* **84**(3): 427-429.
- [34] Price, A.H. and G.A. F., Hendry, 1991. Iron-catalysed oxygen radical formation and its possible contribution to drought damage in nine native grasses and three cereals. *Plant Cell Environ.* **14**: 477-484.
- [35] Shao, H. B.; Chen, X. Y.; Chu, L. Y.; Zhao, X. N.; Wu, G. Y.; Yong, B.; Zhao, C. X. and Z. M., Hu, 2006. Investigation on the relationship of proline with wheat anti-drought under soil water deficits. *Colloids and surfaces. B: Biointerfaces* **53**(1): 113-119.
- [36] Vendruscolo, A. C. G.; Schuster, I.; Pileggi, M.; Scapim, C. A.; Molinari, H. B. C.; Marur, C. J. and L. G. C., Vieira, 2007. Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. *J. Plant. Physiol.* **164**(10): 1367-1376.
- [37] Wang, J. R. and S. X., Li, 2000. Effect of water-limited deficit stress in different growth stages on winter wheat grain yields and their yield constituents, *Acta Bot. Boreal-Occident Sin.* **20**(2): 193-200.
- [38] Wang, W. X.; Vinocur, P. and A., Altman, 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* **218**(1): 1-14.
- [39] Winston, G.W., 1990. Physiochemical basis for free radical formation in cells: production and defenses. *In Stress Responses in Plants: Adaptation and Acclimation Mechanisms*. Edited by Alscher, R.G. and Cumming, J.R. pp. 57-86. Wiley-Liss, Inc., New York.
- [40] Siegel, B. Z., 1993. Plant peroxidases—an organismic perspective. *Plant Growth Regul.* **12**: 303-312.