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RESEARCH ARTICLE

EVALUATION OF OXIDATIVE STRESS TOLERANCE IN TWO DIFFERENT WHEAT CULTIVARS IN RESPONSE TO DROUGHT STRESS

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ABSTRACT

Shortage of water leads to drought stress. Drought stress causes generation of activated oxygen species (AOS) in plants. To overcome water shortage problem plants shows some adaptive mechanism such antioxidant defense and accumulation of osmolytes or osmoprotectants against "AOS". Activity of enzymes such as catalase (CAT), proxidase (POD) and amylase, protein content and accumulation rate of osmoregulants such as proline and carbohydrate were studied in two wheat (*Triticum aestivum L.*) cultivars. The results suggest that water stress increased the activity of enzymes and rate of accumulation of proline and carbohydrate in both cultivars. But out of these two cultivars rain fed cultivar showed significant response during drought stress.

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INTRODUCTION

In nature plants usually get subjected to various biotic and abiotic stresses. Stress is external factor which shows adverse effect on plant growth. Shortage of water leads to drought stress. Plant growth is adversely influenced when plant get exposed to such drought condition. Drought is a major problem which shows more negative impact on crop productivity in arid region more than any other single environmental factor (Boyer, 1982). During drought stress, closure of stomata occurs due to water loss from plant body. In ABA mediated stomatal closure ABA concentration increases.ABA causes alkalization of guard cell cytosol which enhances efflux of anion and K^+. Solute loss also takes place which driving stomatal closure by losing guard cell turgor. Stomatal closure leads to decrease in Co_2 concentration in mesophyll tissue which leads to accumulation of NADPH and decrease in NADP content. Under such condition oxygen act as alternative electron accepter which leads to formation of superoxide radical (O_2^-), H_2O_2 and Hydroxy radical (OH^-) produced by the Haber-Weiss reaction (Egneust *et al*., 1975; Elstner, 1987; Cadenas, 1989). It can cause lipid peroxidation and consequently membrane injury, protein degradation, enzyme inactivation, pigment bleaching and disruption of DNA strands (Fridovich, 1986, Liebler *et al*., 1986, Davies, 1987; Imlay and Linn, 1988). Thus, abiotic stress leads to "AOS" (activated oxygen species) which shows lethal effect at cellular level. Against such drought stress plant undergoes various physiological, morphological, and metabolic or biochemical and developmental changes to cope up with this abiotic stress (Hanson and Hitz, 1982). To overcome water shortage problem plants shows some adaptive mechanism such antioxidant defense and accumulation of osmolytes or osmoprotectants against "AOS". Antioxidants are the molecules which are capable for slowing or preventing the oxidation of other molecules. Enzymes like superoxide dismutase SOD, catalase (CAT), peroxidase (POD), amylase and ascorbic peroxidase (APX) act as an antioxidant.

SOD catalyzes the dismutation of superoxide anion radical (O2~) with great efficiency, resulting in the production of H_2O_2 and O_2 (Smirnoff, 1993; Winston, 1990). Catalase is an oxidoreductase, located in peroxysomes and considered as an important enzyme to counter hydrogen peroxide in stress by reacting with H_2O_2 directly to form water and oxygen (Smirnoff, 1993; Winston, 1990; Srivalli *et al.*, 2003 and Khana-chorpa and Selote, 2007). Catalase is responsible for decomposition and detoxification of H_2O_2 in the peroxisomes. The activity of this enzyme is sensitive to heat as well as drought stress (Jiang and Hoang, 2001). Peroxidase is oxydoreductase enzymatic antioxidant which has one a prostatic group (homogenous-b)and catalysis oxidation of the proton relieving compounds with H_2O_2 and consequently causes H_2O_2 to breakdown (Jiang and Zhang, 2004). Peroxidases catalyze hydrogen peroxide-dependent oxidation of substrates (RH2) according to the general equation.

$$RH_2 + H_2O_2 \longrightarrow 2H_2O$$

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Under drought stress plant shows osmotic regulation. Osmotic regulation is nothing but the regulation of osmotic potential within a cell either through accumulation or through degradation of solutes in plant cell till equilibrium gets obtained. In this phenomenon plant lowers down the osmotic potential of cell for the turgor pressure maintenance (Jones *et al.*, 1981). Thus decrease in the osmotic potential is either through solute accumulation within the plant cell or by a decreased cell volume leading to an increased concentration of osmotic solutes as water leaves from the vacuole. The most common adaptive mechanisms are the accumulation of intracellular solutes like sugars and free amino acids. Most frequent nitrogen-containing compounds that accumulate in plants are amides such as glutamine and asparagine, amino acids like arginine, proline, citrulline, and ornithine, and polyamines like putrescine (Rabe, 1990). During stress, among these solutes proline shows significant increase in its level and accumulation on dehydration of plant cell during drought stress.

Triticum aestivum L. is commonly known as bread wheat which is allohexaploid. It is the basic staple food and an important part of the daily diet of millions of people. India has the largest area in the world under wheat cultivation and its ancestors and close relatives may have potential traits of drought resistance for breeding programs (Shimshi *et al.*, 1982) Main objective of this study is to find the physiological response of two different wheat varieties (*Triticum aestivum L.*)- MACS6222 and NI 5439, under drought stress and this was done by estimating Protein, Proline and total carbohydrate content and by evaluating antioxidant activity such as amylase, POD and CAT under stress condition.

MATERIALS AND METHODS

In the present study, we have selected two different *Triticum aestivum* L. varieties viz. MACS6222 and NI 5439 were selected. The seed material of these varieties was obtained from "Agharkar Research Institute", Pune (Maharashtra). Sowing was done in earthen pots in Randomized Block Design (RBD) manner. Experiment was planned for two different conditions first one is controlled condition that is irrigated one and another one is experimental that is drought stressed condition. For conducting this experiments four replicates of each cultivar including replicates were prepared and except control all were kept under stress. Approximately 20-25 seeds were sown in each pot. After 22 days from sowing and up to 35th day of sowing all pots were irrigated with Hogland's solution, thus it included four treatments of fertilizer. As experiment was designed for two conditions all cultivars are grouped in two sets.

- In first set, controlled pots were regularly irrigated and they were grown under same condition throughout the whole experimental period.
- In second set, experimental pots were drought acclimated by water cessation for 12 days.

After this plants were re-watered and then they were subjected to second drought for 12 days. All measurements were carried out with leaves at same developmental stage and samplings from both set were done. Different physiological parameters and enzyme assay were carried they are as follows:

1. Carbohydrate by anthrone method (Hedge and Hofreiter, 1962).
2. Proline estimation by Chinard method. (Chinard, 1995).
3. Protein estimation by Bradford method (Bradford, 1976).
4. Amylase activity by Peter and Kruger method(Peter,1995 & Kruger, 1972)
5. Catalase activity by Putter & Malik and Singh method (Putter, 1974; Malik and Singh, 1980).
6. Peroxidase activity by Luck method (Luck, 1974).

In present work not only the different enzyme assays such as amylase, catalase and peroxidase and superoxide dismutase were carried out but the estimation of total Carbohydrates, proteins and proline were also estimated to evaluate the antioxidant activity during drought stress. Same experiments were repeated in next year. Data of both years were utilized for statistical analysis and final conclusions were drawn.

RESULTS AND DISCUSSION

Results of proline estimation indicate that water deficit or drought stress condition leads to increase in proline accumulation rate in both varieties as compare to control. Significant increase that is (2.394 & 3.405 µmole/100gm resp.) was found in NI 5439 variety (Table No. II) Higher proline content in wheat plants after water stress has been reported by (Errabii *et al.*, 2006, Patel and Vora, 1985;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 is due to water deficit has been reported by many authors (Delauney and Verma, 1993; Johari-Pireivatlou *et al.*, 2010). The results of protein estimation indicated that both the varieties are not much differed with respect to their "Protein content '' but out of these varieties NI 5439 showed higher degradation of protein i.e. lower value (0.615 & 8.148 µmole/100gm resp.) and lower degradation (higher value) was shown by MACS 6222 (1.329 & 8.600 µmole/100gm resp.) (Table No. II).

Inhibition of protein synthesis induced by water stress (Badiani *et al.* 1990, Price and Hendry, 1991).Contribution of cysteine proteases to total proteolytic activity increases drastically in response to water deficit in wheat (Zagdanska and Winievski, 1996). A significant increase in the carbohydrates values was clearly observed in drought stressed plants compared to control from the booting stage. Wheat plants in the Boot stage showed the highest Carbohydrate contents in NI 5439 (3.793 & 3.420 µmole/100gm resp.) as compare to the control (2.023 & 2.390 µmole/100gm resp.) (Table No. I) Accumulation of soluble carbohydrate increases the resistance to drought to plant. Soluble carbohydrates have role in osmotic regulation and conservation mechanism (Martin *et al.*, 1993).Osmotic stress in plant cells leads to a reduction in carbon assimilation, which is linked to a physiological closure of leaf stomata and to biochemically determined lower photosynthetic activity, which affects carbohydrate economy (Chaves *et al*., 2002). Soluble sugars are acting as osmolytes maintaining cell turgor of

Table No. I: Total carbohydrate content in wheat (First Year & Second Year)

S. No.	First year		First year		Second year		Second year	
	0.5 µmole/gm	1	0.5 µmole/100gm	1	0.5 µmole/gm	1	0.5 µmole/100gm	1
C1	0.0053	0.0088	1.074	1.770	0.044894	0.8978818	0.009706	1.941195
C2	0.0056	0.0101	1.138	2.023	0.060702	1.214037	0.011951	2.390136
E1	0.0116	0.0161	2.339	3.224	0.048372	0.967436	0.013563	2.712615
E2	0.0129	0.0189	2.592	3.793	0.06576	1.315207	0.017104	3.420803

(**C**- Control, **E**- Experimental,**1**-MACS6222,**2**- NI 5439)

Table No. II: Analysis of proline and protein content in the wheat

S.No.	First year		First year		Second year		Second year	
	Protein µmole/gm	Proline	Protein µmole/100gm	Proline	Protein µmole/gm	Proline	Protein µmole/100gm	Proline
C1	0.0499	0.0039	4.993	0.390881	0.0930	0.0069	9.300	0.693
C2	0.0329	0.0092	3.298	0.928342	0.0905	0.0238	9.053	2.384
E1	0.0132	0.0185	1.329	1.856	0.0860	0.0294	8.600	2.941
E2	0.0061	0.0239	0.6152	2.394	0.0814	0.0340	8.148	3.405

Table No III: Activity of amylase enzyme in the wheat

S.No.	Free β-Amylase				Bound β-Amylase			
	units/gm		units/100gm		units/gm		units/100gm	
	I YEAR	II YEAR	I YEAR	II YEAR	I YEAR	II YEAR	I YEAR	II YEAR
C1	14.571	9.2857	1457.14	928.57	3.2857	7.1428	328.57	714.28
C2	14.142	7.428	1414.28	742.8	2.571	2.857	257.14	285.7
E1	20.714	16.428	2071.4	1642.8	6.714	2.148	671.428	214.8
E2	18	14.428	1800	1442.8	5.714	1.285	571.42	128.5

Table No. IV: Activity of peroxidase and catalase enzyme in wheat

S.No.	Activity of Enzymes in units/gm tissue			
	First Year Data		Second Year Data	
	Peroxidase	Catalase	Peroxidase	Catalase
C1	2.4	4.73	7.8	1.488
C2	3	1.35	8.1	1.015
E1	9.3	6.76	15.9	3.383
E2	9	9.47	13.5	4.263

leaves, protecting the integrity of the membrane, and preventing the denaturation of proteins (Mohammadkhani and Heidari, 2008). Enhanced CAT activity was observed in all varieties but NI 5439 wheat variety showed height value (9.47 & 4.263 units/gm resp.) when they exposed to water stress (Table No. IV). During drought stress in wheat (*Triticum aestivum L.*) activity of enzymatic antioxidant CAT is increased to manage the oxidative stress (Mohammad and Mahdiyeh, 2013). The similar results are also obtained in present investigation. Catalase (CAT) reacts with H_2O_2 directly to form water and oxygen (Smirnoff, 1993 & Winston, 1990). Catalase is responsible for decomposition and detoxification of H_2O_2 in the peroxisomes. The activity of this enzyme is sensitive to heat as well as drought stress (Jiang and Hoang, 2001). Present investigation indicates that during water stress all wheat cultivars show increased activity of peroxidase shown in (Table No. IV).But MACS6222 is greater in activity (9.3 & 15.9 units/gm. resp.). An increase of POD activity a 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 wheat had higher POD activity which was reported by (Zang and Khirkham, 1994). Peroxidase is another enzymatic antioxidant system, is an oxydo-reductase that has one homogenous-b as a prostatic group and catalysis oxidation of the proton giver compounds with H_2O_2 and consequently causes breakdown of H_2O_2in to water molecule (Jiang and Zhang, 2004). Amylase shows increased activity in experimental plant as compare to control, but highest value of freer amylase content (2071.4 & 1642.1 units/gm. resp.) in MACS 6222 variety was found. While lowest value was found in variety NI 5439 (1800 & 1442.8 units/gm. resp). MACS 6222 variety shows increased performance for amylase with respect to increased water stress condition.

Highest bound amylase content (671.428 & 214.8 units/gm. resp.) was observed in variety MACS 6222 while variety NI 5439 show low content of bound amylase (571.4 and 128.5units/100gm. resp.) as shown in (Table No. III). These results are consistent with other studies reporting the increased amylase activity in response to drought stress in wheat.

Summary and 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 NI 5439 variety shows highest carbohydrate content in both control as well as experimental condition for both year. Higher proline accumulation was observed in NI 5439 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 these MACS6222 variety showed higher protein content and lower accumulation of carbohydrate and proline in both control as well as experimental plants during both year. MACS6222 variety showed higher peroxidase activity than NI 5439. 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 6222 in both controls as well as in experimental condition. Higher content of bound β-amylase is also found in MACS 6222 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 MACS6222 variety. NI 5439 variety showed significantly increased activity with respect to all parameter in experimental than control conditions for both the year though it is a rain fed variety. From this we can conclude that NI 5439 Variety can show the ability of wheat plants to acclimate under drought stress condition. Further morphological are required to conclude that MACS 2496 variety can be used for cultivation in rain fed area.

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REFERENCES

Badiani, M., De Biasi, M.G., Colognola, M. and Artemi, F. 1990.Catalase, peroxidase and superoxide dismutase activities in seedlings submitted to increasing water deficit. *Agrochimica.*, 34: 90-102.

Boyer, J. S. 1982. Plant productivity and environment. *Science.* 218: 443-448

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.

Cadenas, S. E. 1989. Biochemistry of oxygen toxicity. *Ann. Rev. Biochem*., 59: 79-110.

Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M. L., Ricardo, C.P.P., Osorio, M. L., Carvalho, I., Faria, T. and Pinheiro, C. 2002. How plants cope with water stress in the field. Photosynthesis and growth. *Ann. Bot.,* 89: 907–916.

Chinard, E. P. 1952. Photometric estimation of proline and ornithine. *J. Biol Chem.*,199: 91.

Davies, K. J. A. 1987. Protein damage and degradation by oxygen radicals. I. General aspects. *J. Biol.Chem.,* 262:9895- 9901.

Delauney, A. J. and Verma, D.P.S. 1993. Proline biosynthesis and osmoregulation in plants. *Plant J.,* 4: 215-223.

Dwivedi, S., Kar, M. and Mishra, D. 1979. Biochemical changes in excised leaves of *Oryza sativa* subjected to water stress. *Physiol. Plant.,* 45: 35-40.

Egneust, H.; Heber, U. and Kirk, M. 1975. Reduction of oxygen by the electron transport chain of chloroplasts during assimilation of carbon dioxide. *Biochim. Biophys. Acta.*, 408: 252-268.

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.

Errabii, T.; Gandonou, C. B.; Essalmani, H.; Abrini, J.; Idaomar, M. and Skali-Senhaji, N. 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.

Fridovich, I. 1986. Biological effects of superoxide radical.Arch. *Biochem. Biophys.,* 247: 1-11.

Hanson, A. D. and W. D., Hitz, 1982. Metabolic responses of mesophytes to plant water deficits. Ann. Rev. Plant Physiol., 33: 163-203.

Hedge, J. E. and Hofreiter, B. T. 1962. In: Carbohydrate chemistry, Academic Press, New York : 17

Himshi, D., Mayokal. M. L. And Atsmon, D. 1982. Responses to water stress in wheat and related wild species. *Crop Sci*., 22: 123-1 28.

Imlay, J. A. and Linn, S. 1988. DNA damage and oxygen radical toxicity. *Science,* 240:1302-1309.

Jang, S. 2004. Variation in antioxidant metabolism of young and mature leaves of *Arabidopsis thaliana* subjected to drought. *Plant sci.,* 166: 459-466.

Jiang, Y. and Huang, N. 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.

Johari-Pireivatlou, M., Qasimov, N. and Maralian, H. 2010.Effect of soil water stress on yield and proline content of four wheat lines. *African J. Biotechnol*., 9: 036–040

Jones, M. M.; N. C., Turner and Osmond, C.B. 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.

Khanna –Chopra R. and Selote, D. S. 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.

Kruger, J. E. 1972. Organic and bioorganic chemistry of carbon dioxide. *Cereal Chem.,* 49: 379.

Liebler, D. C.; Kling, D. S. and Reed, D. J. 1986. Antioxidant protection of phospholipid bilayers by α-tocopherol, Control of α-tocopherol status and lipid peroxidation by ascorbic acid and glutathione. *J. Biol. Chem.,* 150: 12114-12119.

Luck, H. 1974. In: Methods in Enzymatic Analysis, 2 (Ed. Bergemeyer), Academic Press, New York: 885.

Malik, C. P. and Singh, M. B. 1980. In: Plant Enzymology and Histoenzymology. Kalayani publisher, New Delhi.

Martin, M., Michell F., Morgan, J. A., Scalet, M. and Zebri, G. 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.

Mohammad, R. A. and Mahdiyeh, M. 2013.Antioxidative and biochemical responses of wheat to drought stress, *ARPN Journal of Agricultural and Biological Science*, 8: 1990-6145

Mohammadkhani, N. and Heidari, R. 2007. Effects of water stress on respiration, photosynthetic pigments and water content in two maize cultivars. *Pakistan J. Biol. Sci*., 10: 4022–4028

Mohammadkhani, N. and Heidari, R. 2008. Drought-induced accumulation of soluble sugars and proline in two maize varieties. *World Appl. Sci .J., s*13: 448–453.

Patel, J. A. and Vora, A. B. 1985. Free proline accumulation in drought stressed plants. *Plant and Soil,* 84(3): 427-429.

Peter B. 1995. In: Methods of Enzymology Academic Press, New York, 1: 149.

Price, A.H. and Hendry, G. A. F. 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.

Putter, J. 1974. In: Methods in Enzymatic Analysis, 2 (Ed. Bergemeyer), Academic Press, New York: 685.

Rabe; E. 1990. Stress physiology: The functional significance of the accumulation of nitrogen-containing compounds. *J. Hort Sci*., 65:231.

Shao, H. B., Chen,X. Y., Chu, L.Y., Zhao, X. N., Wu, G. Y., Yong, B., Zhao, C. X. and Hu, Z. M. 2006. Investigation on the relationship of proline with wheat anti-drought under soil water deficits. Colloids and surfaces*: Bio interfaces,* 53(1): 113-119.

Siegel, B. Z. 1993. Plant peroxidases an organismic perspective. *Plant Growth Regul.*, 12: 303-312.

Smirnoff, N. 1993.The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol.125: 27-58.

Srivalli, B. G.; Sharma and Khanna-Chopra, R. 2003.Antioxidativedefence system in an upload rice cultivar subject to increase intensity of water stress following by recovery. *Physiol. Planta*., 119: 503-512.

Vendruscolo, A. C. G.; Schuster, I.; Pileggi, M.; Scapim, C. A.; Molinari, H. B. C.;Marur, C. J. and Vieira, L. G. C. 2007. Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. *J. Plant. Physiol*., 164(10): 1367-1376.

Wang, J. R. and Li, S. X. 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.

Wang, W. X.;Vinocur, P. and Altman, A. 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta.,* 218(1): 1–14.

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., J.R. Wiley-Liss, Inc.,New York: 57-86.

Zang, J., and Khirkham, M. B. 1994. Drought stress induced change in activities of superoxide dismutase, catalase and peroxidase in wheat species. *Plant Physiol.* 35:785-791.

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