

Studies on Different Physiological parameters under Water Stress Condition in Different Wheat Cultivars

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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 show some adaptive mechanism such as antioxidant defense and accumulation of osmolytes or osmoprotectants against "AOS". Activity of enzymes such as catalase (CAT), peroxidase (POD) and amylase, protein content and accumulation rate of osmoregulators 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 all four cultivars.

Keywords: water stress enzyme wheat

1. Introduction

With global climatic change and increasing shortage of water resources wheat production is greatly influenced (Vasil 2003), Biotic and abiotic stress conditions can give rise to active oxygen species (AOS), resulting in oxidative damage at cellular level. AOS are by-products of aerobic metabolism and their production is enhanced during drought is the limitation of photosynthesis and usually accompanied with formation of AOS in chloroplast such as superoxide radical (O₂⁻), hydrogen peroxide (H₂O₂) and the hydroxyl radical (OH⁻), (Foyer et al., 1994, Asada 1997).

Survival under this stressful condition, depends on the plants ability to perceive the stimulus generate and transmit the signals and initiate various physiological and chemical changes (Shao et al., 2005). This antioxidant defense appear to provide protection against oxidative damage in cellular membranes and organelles in plants grown under unfavorable conditions.

Acclimation of plants to drought is considered to promote antioxidant defense system to face the increased level of AOS, which in turn cause membrane damage by lipid peroxidation. Antioxidative enzymes like SOD, catalase and Ascorbic peroxidase have been related with water deficiency and are considered as main component of anti-oxidant machinery for drought resistance in plants.

Main objective of this study is

- To study and identify different mechanisms by which drought stress decreases plant growth.
- To study important antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT) and Ascorbic Peroxidase (APX) and their role in scavenging AOS.

2. Materials and Methods

2.1 Plant Material

Four different Wheat cultivars were selected for the present study. These are *Triticum Aestivum* varieties. The seed material for the experiment is obtained from Genetics

Department of Agharkar Research Institute. The varieties we have selected are either suitable for rainfed environment or for irrigated environment. The varieties we have selected are given in Table no.1.

Table 1: List of Varieties

S. No.		Triticum Aestivum	Suitable for
1	C1	MACS2496	Irrigated
2	C2	MACS6222	Irrigated
3	C3	MACS 6145	Rainfed
4	C4	NI 5439	Rainfed

2.2 Experimental Design

The experiment was conducted in earthen pots. The cultivars selected were planted in Randomized Complete Block Design (RBD). The experiment was planned in two different conditions with four replications per genotype. The two different conditions are controlled condition i.e. irrigated one and another one subjecting to drought stress i.e. water stress. Approx. 15 seeds will be sown in each pot. These are also kept under controlled condition.

After 15 days from sowing, the pots are irrigated with half strength of Hoagland solution only up to twenty eight days then the pots for each cultivar were grouped into two sets. In the first set, control (non stressed) plants were grown under these conditions throughout the whole experimental period. In the second set, plants are drought pretreated (drought acclimated) by cessation of watering for 8 days. After this drought period, the same plants were re-watered for 48 h and then subjected to second drought period extended for 12 days. All measurements will be carried out with the leaves at the same developmental stage.

2.3 Measurements

The parameters studied during this experiment are as follows:

- Total Carbohydrate
- Protein estimation
- Proline estimation
- Amylase assay
- Peroxidase and

6. Catalase

- Carbohydrate by anthrone method (Hedge and Hofreiter, 1962).
- Proline estimation by Chinard method. (Chinard, 1995).
- Protein estimation by Bradford method (Bradford, 1976).
- Amylase activity by Peter and Kruger method (Peter, 1995 & Kruger, 1972)
- Catalase activity by Putter & Malik and Singh method (Putter, 1974 & Malik and Singh, 1980).
- 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.

3. Result and Discussion

The results obtained during this study were summarised as follows:

3.1 Carbohydrate Content

A significant increase in the carbohydrates values was clearly observed in stressed plants compared to control from the booting stage. In Aestivum wheat MACS 2496 variety showed highest carbohydrate content (2.52 and 2.53mg/100gm resp.) under control condition. On the other hand under stress condition it showed highest value in MACS 6222 and NI 5439 resp as shown in Table 1.

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 leaves, protecting the integrity of the membrane, and preventing the denaturation of proteins (Mohammadkhani and Heidari, 2008). Sucrose plays an important role in plant metabolism at both cellular and whole organism level. It participates not only in the response to abiotic stresses, but also serves as a nutrient and signalling molecule, modulating a wide range of gene activity (Gibson, 2005).

Table 1: Mean Total carbohydrate content in the wheat

Sr. No.	AESTIVUM (mg/100gm)	
	2012-2013	2014-15
C1	2.52	2.53
C2	2.46	2.47
C3	2.27	2.28
C4	2.46	2.47
E1	2.40	2.40
E2	2.46	2.47

E3	2.40	2.40
E4	2.46	2.47

3.2 Protein Content

The results of protein estimation indicated that the varieties selected are not much differ in their protein content. In Aestivum wheat the highest protein content was observed under MACS 6222 (5.93 and 5.90 mg/100gm resp.) for both the years and similar trend was observed under stress condition. The protein content was decreased under stress condition.

Inhibition of protein synthesis induced by water stress (Badianiet *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 Wi nievski, 1996).

It is established that water deficit stress induces the expression of many genes among which are some genes coding proteases (Bray, 2002; Cruz de Carvalho *et al.*, 2001). Intracellular proteases have an important role in the degradation of damaged or unnecessary proteins, metabolism reorganisation and nutrient remobilization under stress (Feller, 2004; Grudkowska and Zagdanska, 2004). It is important for the agricultural practice to understand the relation between proteolysis and plant performance in drought conditions and recovery from stress (Chaves and Oliveira, 2004). It is not clear whether high proteolytic activity under stress conditions is advantageous for the plant allowing reorganization of protein pattern or it leads to cell disintegration (Zagdańska and Wi nievski, 1996). Some experimental evidence suggests that drought sensitive species and varieties have higher proteolytic activity compared to resistant ones (Roy-Macauley *et al.*, 1992; Zagdanska and Wi nievski, 1996; Hienget *al.*, 2004).

Table 2: Mean Total Protein content in the wheat

S. No.	AESTIVUM (mg/100gm)	
	2012-2013	2014-2015
C1	5.55	5.60
C2	5.93	5.90
C3	5.49	5.50
C4	5.14	5.10
E1	4.85	4.80
E2	4.15	4.60
E3	4.40	4.50
E4	4.05	4.40

3.3 Proline Content

Results indicates that water deficit or drought stress condition leads to increase in proline accumulation rate in all varieties. In Aestivum wheat also the highest value for proline content was observed in MACS 6222 in control as well as stress condition. Similar trend was observed in both durum and Aestivum wheat varieties for both the years as shown in Table 3.

Table 3: Mean Total Proline content in the wheat

Sr. No.	AESTIVUM (umol/100gm)	
	2012-2013	2014-2015
C1	2.39	2.12

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 (Smirnov 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 osmoregulators.

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 osmoregulators 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.

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