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RESEARCH ARTICLE

EVALUATION OF OXIDATIVE STRESS TOLERANCE IN 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 different Durum Wheay 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

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.

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Main objective of this study is

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

MATERIALS AND METHODS

Plant Material

Four different Wheat cultivars were selected for the present study. These are Triticum Durum 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 1.

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.

Table 1. List of Varieties

S.No.		Triticum Durum	Suitable for
1	C1	MACS 1967	Rainfed
2	C2	MACS 3125	Irrigated
3	C3	NIDW 295	Irrigated
4	C4	A-9-30-1	Rainfed

`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.

Measurements

The parameters studied during this experiment are as follows:

1. Total Carbohydrate
2. Protein estimation
3. Proline estimation
4. Amylase assay
5. 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).
- Amylaseactivityby Peter and Kruger method (Peter,1995 and Kruger, 1972)
- Catalase activity by Putter and Malik and Singh method (Putter, 1974 & Malik and Singh, 1980).
- Peroxidase activity byLuck 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.

RESULT AND DISCUSSION

The results obtained during this study were summarised as follows:

Carbohydrate content

A significant increase in the carbohydrates values was clearly observed in stressed plants compared to control from the booting stage. Wheat plants in the Boot stage showed the highest Carbohydrate contents in A-9-30-1 durum wheat variety (2.99, 3.09mg/100gm) under control conditron and under stress condition it showed highest value in MACS 1967(3.54mg/100gm) and A-9-30-1 (3.31mg/100gm) for both the years. 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

S. No.	DURUM (mg/100gm)	
	2012-2013	2014-2015
C1	2.65	2.71
C2	2.80	2.97
C3	2.74	2.84
C4	2.99	3.09
E1	3.54	3.22
E2	3.17	3.28
E3	3.17	3.16
E4	3.31	3.41

Protein content

The results of protein estimation indicated that the varieties selected are not much differ in their protein content. In durum wheat varieties MACS 3125 (5.70, 5.90 mg/100gm resp.) showed higher protein content as compared under stress condition for both the years. Under stress condition the protein content decreases as compared to control as shown in Table 2. 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 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 (Zagdañska 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 Winievski, 1996; Hieng *et al.*, 2004).

Table 2. Mean Total Protein content in the wheat

S.No.	DURUM (mg/100gm)	
	2012-2013	2014-2015
C1	5.45	5.76
C2	5.70	5.90
C3	5.35	5.40
C4	5.23	5.20
E1	4.30	4.40
E2	4.58	4.60
E3	4.60	4.80
E4	4.58	4.60

Proline content

Results indicates that water deficit or drought stress condition leads to increase in proline accumulation rate in all varieties .In durum wheat the highest value was observed in A-9-30-1 under control as well as under stress condition.

Table 3. Mean Total Proline content in the wheat

S. No.	Durum (umol/100gm)	
	2012-2013	2014-2015
C1	2.12	2.94
C2	2.17	2.60
C3	1.81	1.92
C4	2.92	3.15
E1	3.15	3.46
E2	3.39	3.43
E3	2.10	2.25
E4	3.46	3.37

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). Manyreports 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 and 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.

Amylase Activity

Amylase shows increased activity in experimental plant as compare to control. In durum wheat highest free β-amylase value was observed under stress condition in A-9-30-1 (981 and 1000 units/gm resp.) for both the years. Also higher bound β-amylase value was observed under MACS 1967 (185 units/gm) and MACS 3125 (198.5 units/gm) for both consecutive years as shown in table no.4. These results are consistent with other studies reporting the increased amylase activity in response to drought stress in wheat (Bakalova *et al.*, 2004 and Csiszar *et al.*, 2005)

Catalase enzyme activity (CAT)

Enhanced catalse enzyme activity was observed in MACS 3125 and A-9-30-1 for both years in durum wheat (3.7 and 4.2 units/gm) as compared to control. Results are shown in table no.4. Similar trend 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 and 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 (Srivalli *et 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

Table 4. Mean of different enzyme activities in Durum wheat

S. No.	Durum Wheat (2012-2013)				Durum Wheat (2013-2014)			
	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	928.57	158.57	1.7	2.41	828.60	178.57	2.5	2.030
C2	814. 28	128.55	2.4	2.73	714.30	138.57	28	1.488
C3	960.00	125.54	1.9	2.70	957.14	145.71	2.2	1.353
C4	871.42	150.71	2.3	2.35	971.40	155.71	2.1	1.015
E1	1014.28	185.71	2.2	3.52	914.30	191.43	3.2	3.654
E2	900.00	157.14	2.8	3.76	800.00	198.57	3.7	3.383
E3	961.42	160.85	2.6	3.44	971.40	162.86	3.4	3.586
E4	981.20	167.14	3.2	3.47	1000.00	187.14	3.2	4.263

sensitive to H2O2. A high level of H2O2 directly inhibits CO2 fixation (Yamazaki *et al*., 2003). 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). 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 H_2O_2 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 (Dat *et al.*, 1998).

Peroxidase

In the present study, during water stress all wheat cultivars showed increased activity of peroxidise enzyme as shown in (Table 4 and 5). In present study ,in durum wheat highest peroxidise activity as compared to control was found in A-9-30-1 and MACS 3125(3.2 and 3.7 units/gm resp.) for both the years under stress condition. Similar trend was observed for Aestivum wheat under water stress condition. In Aestivum wheat MACS 6222 and NI 5439 showed highest peroxidise enzyme activity as compared to control as shown in Table 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).

Conclusion

Present study is initiative for studying the different mechanisms under drought stress conditions. With the global climatic change and increase in shortage of water, there is continuous decrease in wheat production. Acclimation of plants to drought 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, in durum wheat A-9-30-1 variety showed highest carbohydrate content in both control as well as experimental condition for both year. Higher proline accumulation was observed in A-9-30-1 variety and it also showed low protein content, this may be due to protein degradation during stress and this degradation might be leads to increase in proline content. During water stress condition proline and carbohydrates are act as osmoregulants. In durum wheat A-9-30-1 and MACS 3125 showed increase enzymatic activity and in Aestivum wheat MACS 6222 and NI 5439 showed higher enzyme activity in amylase, peroxidise and catalase enzyme. From the results obtained during this study we can say that the different varieties showed systematic increase in enzyme activity particularly peroxidase and catalase under drought stress condition. Out of these four Durum varieties A-9-30-1 and MACS 3125 and from Aestivum varieties NI 5439 and MACS 6222 showed optimal activity for all the parameters in our study. From this we can conclude that these varieties can show the ability of wheat plants to acclimate under drought stress condition and have ability to withstand under water stress condition. These can be used where there is a scarcity of water. These results can be validated by taking the field trials of these varieties. Further studies are needed.

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