Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 3253-3259
Received: 12-09-2020
Accepted: 17-10-2020

Dommalapati Sudhakara Rao
Department of Chemistry and Biochemistry, Chaudary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Midathala Raghavendra
Department of Chemistry and Biochemistry, Chaudary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Parveen Gill
Department of Zoology, Chaudary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Shashi Madan
Department of Chemistry and Biochemistry, Chaudary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Renu Munjhal
Department of Genetics and Plant Breeding, Chaudary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Corresponding Author:
Dommalapati Sudhakara Rao
Department of Chemistry and Biochemistry, Chaudary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Differential response of anti oxidant system during grain development in drought tolerant and drought sensitive varieties of wheat (*Triticum aestivum* L.)

Dommalapati Sudhakara Rao, Midathala Raghavendra, Parveen Gill, Shashi Madan and Renu Munjhal

Abstract
The present study showed the effect of drought stress on antioxidant enzymes in leaves and developing grains of two wheat varieties *viz*. WH 1105 and WH 1025. The results showed that during grain development, activities of antioxidant enzymes *viz*. Superoxide Dismutase (SOD), Catalase (CAT), Peroxidase (POX), Ascorbate Peroxidase (APX) and Glutathione Reductase (GR) increased more in WH 1025 under drought stress than drought susceptible wheat WH 1105. The increased antioxidant enzyme activities under drought stressed wheat variety WH 1025 in leaf and grain samples during development were due to efficient functioning of the system to cope with drought conditions and made the crop plant tolerant.

Keywords: Wheat *Triticum aestivum* L. drought stress, antioxidant enzymes

Introduction
Wheat (*Triticum aestivum* L.) is the most widely cultivated food crop. In India, it is second important staple food crop after rice. It contributes more calories and protein to the world diet than any other cereal crop. Abiotic stresses such as drought, soil salinity and extreme temperatures adversely affect the productivity and quality of Wheat. Drought stress is one of the major limitations to crop productivity.

Drought stress adversely affects plants by producing reactive oxygen species (ROS), causing membrane integrity loss, damaging proteins, DNA and lipids, and potentially disrupting cell function. In plants, the link between ROS production and defense mechanisms are particularly important. Plants posses very efficient enzymatic and non enzymatic antioxidant defense systems which work in concert to control cascade of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging ROS. Tolerance to abiotic stress is very complex due to intricate interactions between stress factors and various molecular and biochemical phenomenon affecting plant growth and development. Keeping this in view, the present work is planned to study the antioxidant system related drought stress.

Plants possess very efficient enzymatic and non enzymatic antioxidant defence systems which work in concert to control the cascade of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging of ROS (Kyung-Hee *et al.* 2005; Sarvajeet & Narendra, 2010; Abdullah *et al.* 2011) [16, 2]. Several enzymes can efficiently detoxify ROS such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and the enzymes of ascorbate-glutathione cycle (Foyer & Halliwell, 1976) [8] such as ascorbate peroxidase (APX), glutathione reductase (GR), monodehydro ascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) (Goudarzi & Pakniyat 2009; Mostafa *et al.* 2011; Ezzat-Ollah *et al.* 2007) [9, 12, 7]. The non enzymatic protective mechanism is vital in plants to eliminate or reduce ROS, which are effective at different levels of stress induced deterioration (Beak & Skimmer, 2003) [4]. The capacity of cellular antioxidative and photo protective defence is determined by the pool size of the antioxidants and protective pigments and any alteration to these parameters could reflect the impact of stress on plant metabolism. Furthermore, the antioxidant capacity is dependent on stress severity. Among the non enzymatic antioxidants glutathione and ascorbate are most important soluble antioxidants. Requirement of glutathione is for keeping ascorbate in reduced form and to enable cell to maintain the percentage of sulfhydryl groups in thylakoid proteins during rehydration. Increased GSH/GSSG ratio is required for ascorbate regeneration and activation of several

CO_2 fixing enzymes in chloroplast (Zong-Bo *et al.* 2008) [20]. It can enhance gene expression of active oxygen species scavenging enzymes and implicated as an elicitor of several genes related to stress tolerance (Sarika *et al.* 2005) [17]. Carotenoids directly deactivate singlet oxygen and can also quench the excited triplet state of chlorophyll thereby indirectly reduce the formation of active oxygen species (Agarwal *et al.* 2005; Biljana & Stojanovic, 2005) [3, 5].

Materials and Methods

Seeds of two Wheat varieties WH 1105 (Drought sensitive) and WH 1025 (Drought tolerant) were sown in micro plots of Chaudhary Charan Singh Haryana Agricultural University, Hisar farm for drought and irrigated conditions. Drought was created in the field by exclusively giving pre sown irrigation where as under irrigated conditions; recommended irrigations were given at different stages of crop growth and allowed plants to grow. Normal agronomical practices were followed. Plants were tagged and grain samples were collected at four stages starting from the day after anthesis (7, 14, 21 and 28) at an interval of seven days during the period of grain development. Leaf and grain extracts were prepared and were used for antioxidant enzymatic studies, MDA and H_2O_2. Standard protocols were used for assaying the antioxidant enzymes and metabolites.

The whole procedure for the preparation of enzyme extract was done at 0-4 °C. The leaf and developing grain materials were collected from the field, packed in polyethylene bags and buried in crushed ice in thermo cool box and brought to the laboratory. The samples were freed of foreign material by washing thoroughly with tap water followed by distilled water and blotted to dry. Leaf and grain samples weighing 1 g were then homogenized in 4 ml of 0.1M phosphate buffer (pH 7.0) which contains 1% polyvinyl pyrrolidine in a previously chilled mortar and pestle using liquid nitrogen. The homogenate, thus obtained, was centrifuged at 10,000 × g for 20 min. in a refrigerated centrifuge at 4 °C. The supernatant thus so obtained was referred as crude extract and stored in a refrigerator at -20 ºC for enzyme assays. These crude extracts were used for determining the activity of Superoxide Dismutase (SOD), Ascorbate Peroxidase (APX), Glutathione Reductase (GR), Catalase (CAT) and Peroxidase (POX).

Superoxide dismutase (SOD) (EC 1.15.1.1)

Superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) adopting the method of Beauchamp & Fridovich, (1971). The reaction mixture (3.0 ml) contained 50 mM Tris-HCl (pH 7.8), 14 mM L-methionine, 60 mM NBT, 3 mM riboflavin, 0.1 mM EDTA and 0.1 ml of enzyme extract. Riboflavin was added at the end. The tubes were shaken properly and placed 30 cm below light source consisting of three 20W fluorescent lamps (Phillips, India). The reaction was started by switching on light and terminated after 40 min. of incubation by switching off light. After terminating the reaction, the tubes were covered with black cloth to protect them from light. A non-irradiated reaction mixture that did not develop colour served the control. The reaction mixture without enzyme extract developed maximum colour and its absorbance decreased with the addition of enzyme. The absorbance was recorded at 560 nm. Per cent inhibition was calculated by following formula of Asada *et al*. (1974) [6]:

$$\text{Per cent inhibition} = \frac{(V - v)}{v} \times 100$$

Where

V = Rate of assay reaction in absence of SOD.

v = Rate of assay reaction in presence of SOD.

One enzyme unit is defined as the amount of enzyme that inhibits the nitro blue tetrazolium photoreduction by 50%.

Ascorbate peroxidase (APX) (EC 1.11.1.1)

The enzyme activity was assayed by the method of Nakano & Asada (1981) [13] following the oxidation of ascorbic acid. The reaction mixture (2.7 ml) contained 2.25 ml of 100 mM phosphate buffer (pH 7.0), 0.2 ml of 0.5 mM ascorbate, 0.2 ml of 0.1 mM H_2O_2 and 0.05 ml of enzyme extract. The reaction was initiated by the addition of H_2O_2. The decrease in absorbance at 290 nm was recorded spectrophotometrically which correspond to oxidation of ascorbic acid. The enzyme activity was calculated using the molar extinction coefficient of 2.8 mM^{-1} cm^{-1} for ascorbic acid. One enzyme unit is defined as 1 μ mole of ascorbic acid oxidized per min. at 290 nm.

Glutathione reductase (GR) (EC 1.6.4.2)

Glutathione reductase was assayed by using the method of Halliwell & Foyer (1978) [11]. The reaction mixture (3 ml) consisted of 2.5 ml of 0.1 M potassium phosphate buffer (pH 7.5), 0.2 ml EDTA, and 0.15 ml of 50 mM oxidized glutathione (GSSG), 0.1 ml of 30 mM NADPH and 0.05 ml enzyme extract. Initiated the reaction by adding NADPH and monitored decrease in absorbance at 340 nm against 3.0 ml of 0.1 M potassium phosphate buffer (pH 7.5) as blank. An extinction coefficient of 6.22 mM^{-1} cm^{-1} for NADPH was used to calculate the amount of NADPH oxidized which correspond to GR activity. One enzyme unit is defined as 100 nmole of NADPH oxidized per min.

Catalase (CAT) (EC 1.11.1.6)

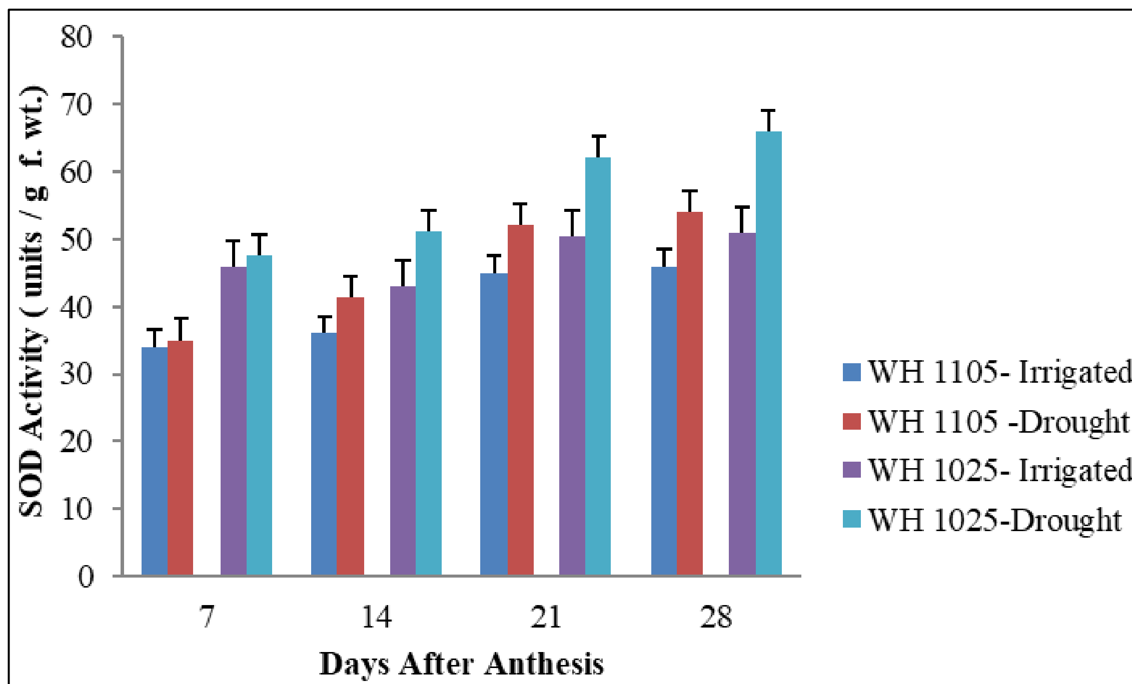
Catalase activity was measured according to the method of Sinha (1972). The reaction mixture (1.0 ml) consisted of 0.5 ml of 0.1 M phosphate buffer (pH 7.0), 0.4 ml of 0.2 M hydrogen peroxide and 0.1 ml of properly diluted enzyme extract. The reaction mixture was incubated at 37 °C and the reaction was terminated by adding 3 ml mixture of 5% (w/v) potassium dichromate and glacial acetic acid (1:3 v/v) to the reaction mixture. The tubes were heated in boiling water bath for 10 min. A control was run under similar conditions where enzyme extract was added after stopping the reaction. After cooling the tubes, absorbance of test and control was measured at 570 nm. The amount of residual H_2O_2 in the reaction mixture was determined by subtracting the absorbance of test samples from that of control. One unit of enzyme activity is defined as the amount of enzyme which catalyzed the oxidation of 1 μ mole H_2O_2 per min. under assay conditions.

Peroxidase (POX) (EC 1.11.1.7)

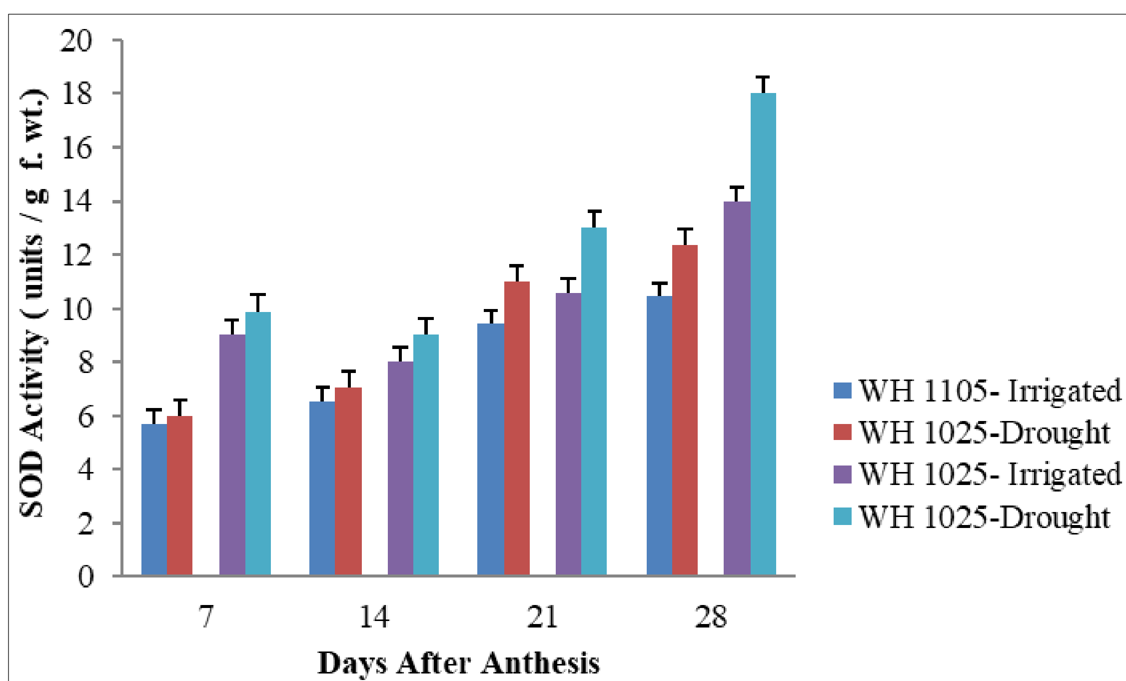
The enzyme was assayed by adopting the method of Shannon *et al.* (1966) [18]. The reaction mixture (2.8 ml) consisted of 2.55 ml of 50 mM phosphate buffer (pH 6.5), 0.1 ml of 0.5% hydrogen peroxide, 0.1 ml of 0.2% ortho-dianisidine and 50 μl of enzyme extract. The reaction was initiated by the addition of 0.1 ml of H_2O_2. The assay mixture without H_2O_2 served as blank. Change in absorbance was recorded at 430 nm for 3 min. at 15 sec. interval. One unit of peroxidase represents 1.0 O.D. change per min.

Results and Discussion
Superoxide dismutase (SOD)
Superoxide dismutase activity is given in Fig.1 (A and B). Under drought stress, SOD activity increased from 7th to 28th days after anthesis over irrigated condition of both the wheat varieties in leaves and developing grains. In leaves of WH 1105, the maximum SOD activity of 17.15 per cent was observed at 28th day after anthesis. The per cent increase in activity showed an increasing trend from 7th to 28th days after anthesis under drought stress condition. While in leaves of WH 1025 the maximum (29.60 per cent) increase of SOD activity was observed at 28th day after anthesis under drought stress over irrigated condition. The enhancement trend of SOD is quite similar to WH 1105.

In developing grains of both varieties, the SOD activity increased from 7th to 28th days after anthesis under drought stress condition compared to irrigated condition (Fig. B). The maximum per cent increase (18.24) was recorded at 28th day after anthesis. The per cent enhancement gradually increased from 7th to 28th days after anthesis in both varieties. The basal level of SOD activity was considerably high and maintained during grain development.



(A)



(B)

Fig 1: Effect of drought stress on superoxide dismutase activity in leaves (A) and developing grains (B) of wheat at different days after anthesis

Ascorbate peroxidase (APX)
Perusal of data in Fig. (2A) shows that APX activity was more under drought stress condition compared to irrigated condition in leaves of WH 1105 and WH 1025 wheat varieties.

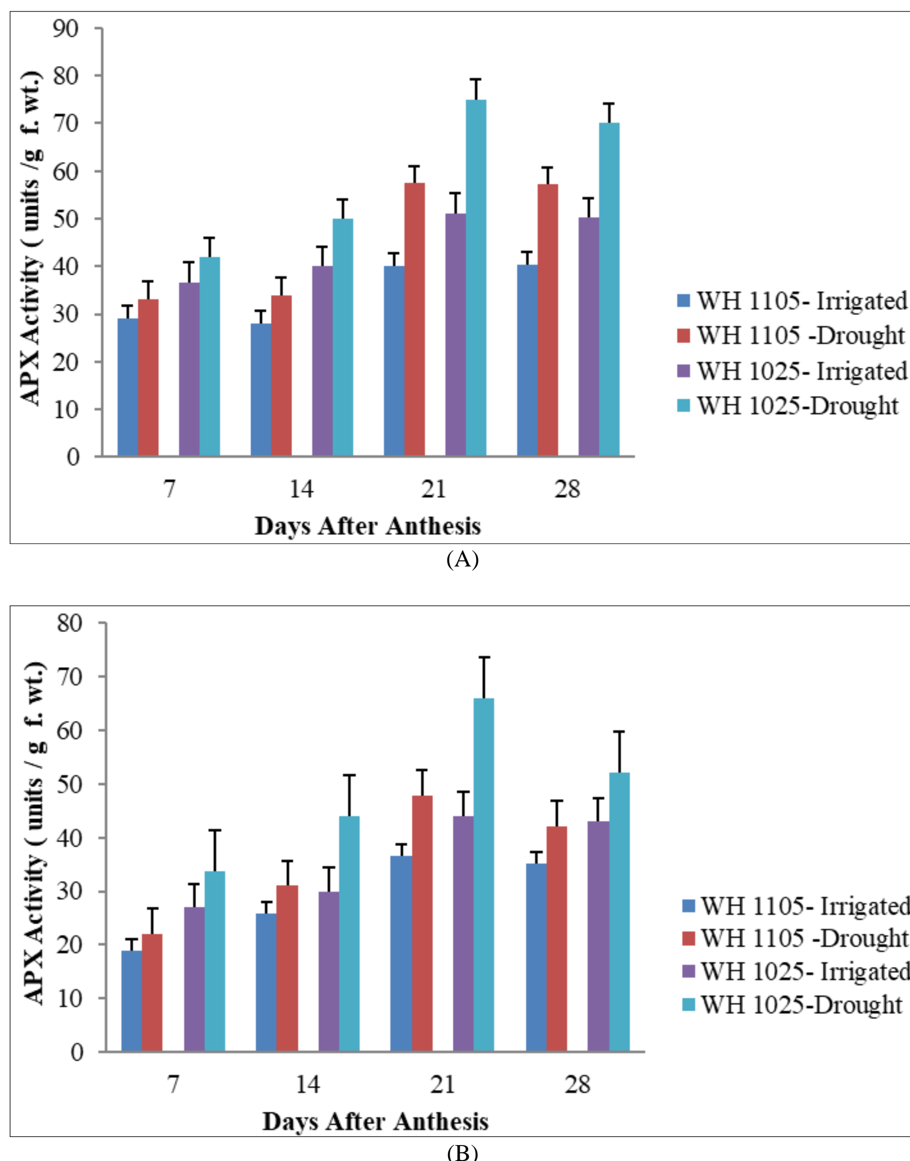


Fig 2: Effect of drought stress on ascorbate peroxidase activity in leaves (A) and developing grains (B) of wheat at different days after anthesis

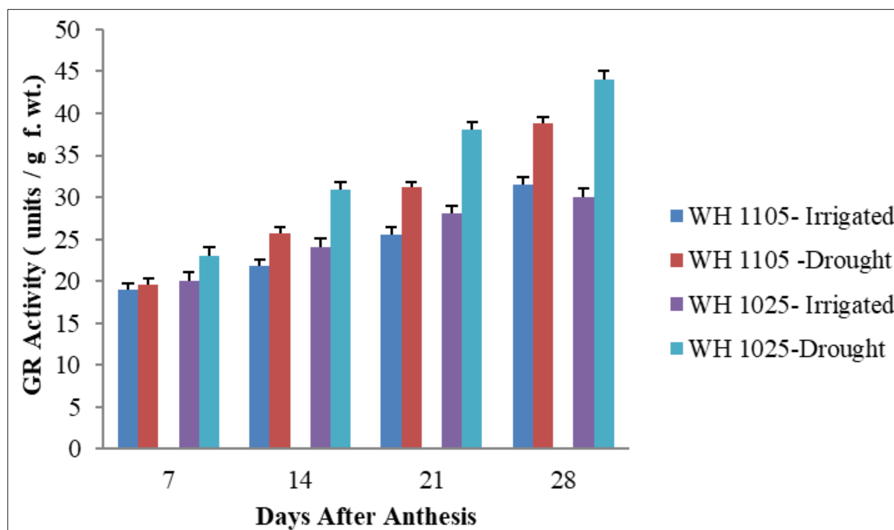
The activity enhanced under drought stress and the level of enhancement varied from 14.44 to 43.92 per cent during different days after anthesis in WH 1105, while the level of enhancement in WH 1025 varied from 14.57 to 46.87 per cent. In leaves of both varieties increase in activity was peaked at 21st day after anthesis and then declined at 28th day after anthesis.

Similarly in developing grains, APX activity enhanced under drought stress condition over irrigated condition (Fig. 2B). The level of enhancement in WH 1105 ranged from 15.84 to 30.43 per cent during different days after anthesis while in WH 1025 the level of enhancement ranged from 20.93 to 50.00 per cent. The activity increased from 7th to 21st days after anthesis and later declined at 28th day after anthesis. The basal level of APX activity was less in WH 1105 compared to WH 1025 during all stages of grain development, thus indicated that response of WH 1025 was more pronounced under drought stress.

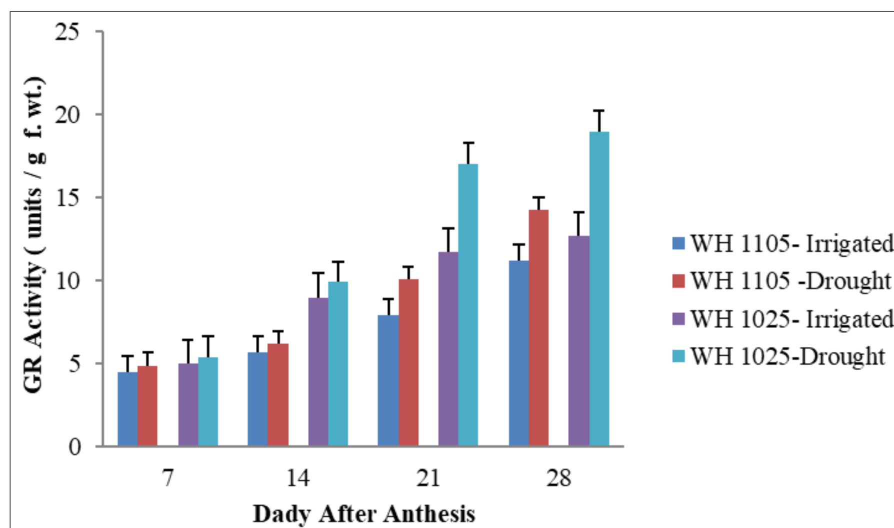
Glutathione reductase (GR)

Results in Fig. 3 (A and B) show the activity of glutathione reductase in leaves and developing grains of both wheat varieties. The GR activity in leaves of both the wheat varieties was substantially more under drought stress condition than that of irrigated condition at different stages of grain development. In leaves of WH 1105, the per cent increase was maximum (23.27) at 28th day after anthesis and minimum (3.53) at 7th day after anthesis. Glutathione reductase activity showed an increasing pattern from 7th to 28th days after anthesis. However the magnitude of increase was more in leaves of WH 1025 that showed maximum enhancement (46.67 per cent) at 28th day after anthesis and minimum (14.86 per cent) at 7th day after anthesis (Fig. 5A). The per cent increase in GR activity in leaves of WH 1025 showed a pattern of enhancement from 7th to 28th days after anthesis.

Maximum (27.02 per cent) increase in GR activity was at 28th day after anthesis and minimum (8.06 per cent) at 7th day after anthesis with an increasing trend from 7th to 28th days after anthesis in developing grains of WH 1105 was observed, while in WH 1025 the maximum (49.49 per cent) of GR activity was at 28st day after anthesis and minimum (8.40 per cent) at 7th day after anthesis with similar trend from 7th to 28th days after anthesis (Fig. 3B). The GR activity was higher at all stages of grain development in WH 1025 in both leaves and developing grains as compared to WH 1105 indicated that WH 1025 had adopted better to the progressive drought stress.



(A)

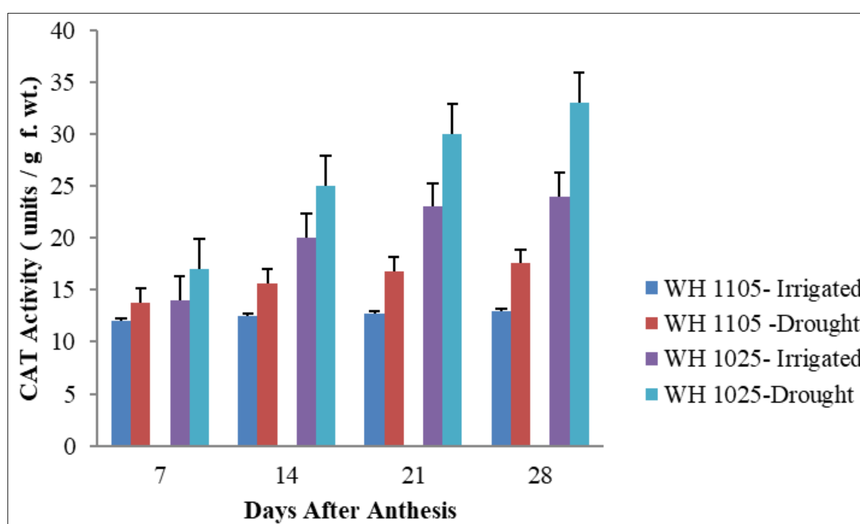


(B)

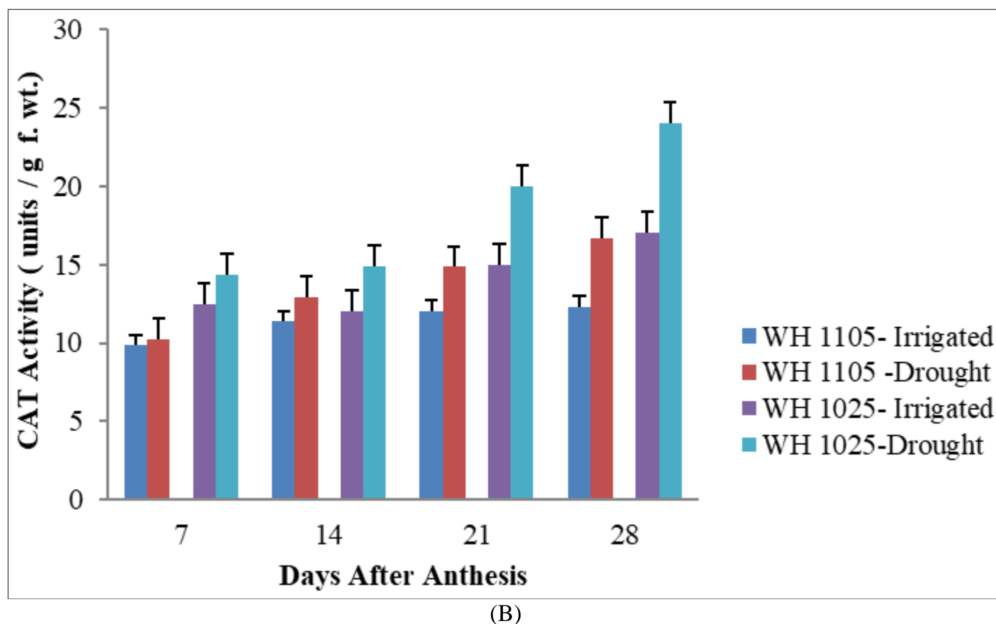
Fig 3: Effect of drought stress on glutathione reductase activity in leaves (A) and developing grains (B) of wheat at different days after anthesis

Catalase (CAT)

Result in Fig. 4 (A and B) shows catalase activity in leaves and developing grains of both wheat varieties under irrigated and drought stress conditions. CAT activity increased under drought stress in leaves and developing grains of both the wheat varieties from 7th to 28th days after anthesis. WH 1105 showed an increasing pattern during different developmental stages and the per cent increase in activity was maximum (35.22) at 28th day after anthesis (Fig. 4A). WH 1025 showed an increasing pattern from 7th to 28th days after anthesis and the per cent increase was maximum (37.50) at 28th day after anthesis. Though there was increase in CAT activity, but enhancement was not much prominent.



(A)



(B)

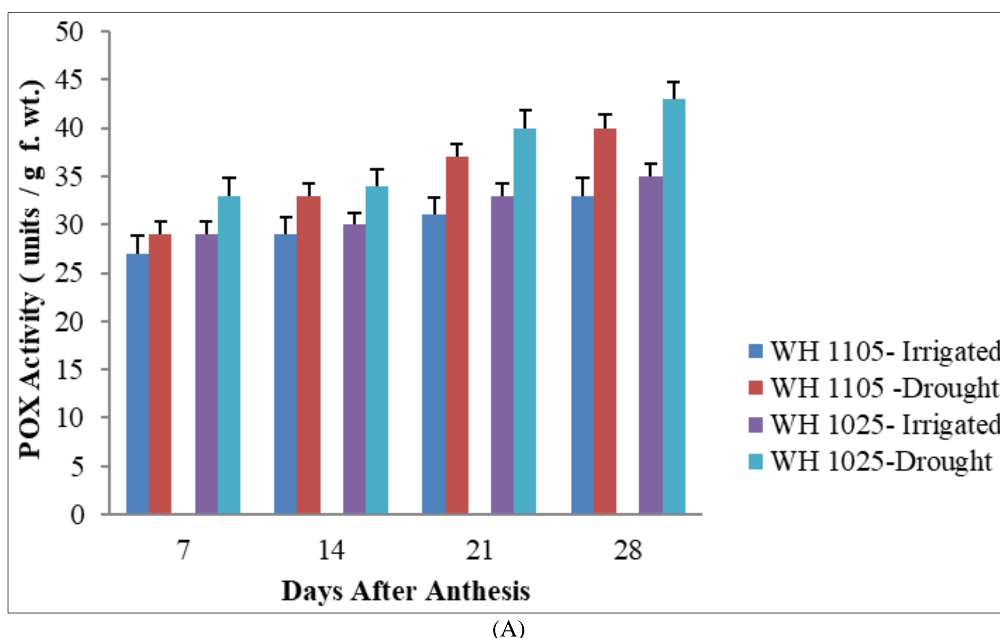
Fig 4: Effect of drought stress on catalase activity in leaves (A) and developing grains (B) of wheat at different days after anthesis

In developing grains of WH 1105, the catalase activity increased under drought stress condition compared to irrigated condition with respect to various grain developmental stages (Fig. 4B). The catalase activity in grains followed similar pattern to that of leaf with maximum (35.20) activity at 28th day after anthesis and an increasing trend from 7th to 28th days after anthesis. Similarly in WH 1025 the maximum (41.18) increase was noted at 28th day after anthesis and trend similar to WH 1105 was noticed.

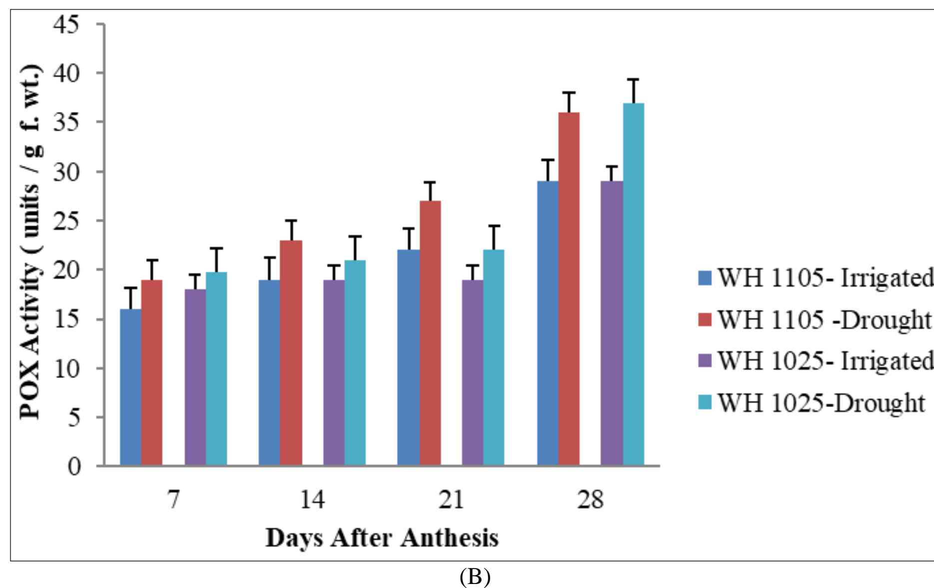
Peroxidase (POX)

The activity of POX increased in leaves and developing grains of both wheat varieties under drought stress condition compared to irrigated condition during all stages of grain development (Fig. 5A). The maximum per cent increase (21.21) was noted at 28th day after anthesis and minimum (7.41) at 7th day after anthesis and showed a pattern in such a way that the POX activity increased from 7th to 28th days after anthesis. While in leaves of WH 1025, POX activity increased under drought stress with a maximum per cent increase (22.86) was noted at 28th day after anthesis and minimum (13.79) at 7th day after anthesis. Slightly higher activity of POX was recorded in leaves of WH 1025 compared to WH 1105.

POX activity in developing grains increased under drought stress. In WH 1105, maximum (24.14 per cent) increase was noted at 28th day after anthesis and minimum (18.63 per cent) at 7th day after anthesis and showed an increasing trend from 7th to 28th days after anthesis (Fig. 3B). While in WH 1025, maximum (27.59 per cent) POX activity was observed at 28th day after anthesis and minimum (10.00 per cent) at 7th day after anthesis and showed increasing trend from 7th to 28th days after anthesis. Comparing the POX activity in developing grains of both wheat varieties, WH 1025 had shown slightly higher activity than WH 1105.



(A)



(B)

Fig 5: Effect of drought stress on peroxidase activity in leaves (A) and developing grains (B) of wheat at different days after anthesis

Conclusions

1. The activities of antioxidant enzymes were increased under drought condition compared to irrigated condition in both sensitive and tolerant varieties.
2. The extent of increase in the antioxidant enzymes was more in drought tolerant variety than drought susceptible variety.
3. The better tolerance character of WH 1025 during present investigation might be due to the enhanced activities of antioxidant enzymes involved in scavenging active oxygen species.

References

1. Abdullah A, Al-Ghamdi. Evaluation of antioxidant stress tolerance in two wheat (*Triticum aestivum*) cultivars in response to drought. Intl. J. Agril. Biol. 2009;11:7-12.
2. Abdullah M, Davood H, Mahyar R, Saeed M. Effects of drought stress on antioxidant enzymes activity of some chickpea cultivars. American-Eurasian Journal of Agricultural and Environmental Sciences 2011;11(6), 782-785.
3. Agarwal S, Sairam RK, Srivastava GC, Meena RC. Changes in antioxidant enzymes activity and oxidative stress by abscisic acid and salicylic acid in wheat genotypes. Biologia Plantarum 2005;49(4):541-550.
4. Beak KH, Skimmer DZ. Alterations of antioxidant enzyme gene expression during cold acclimation of near-isogenic wheat lines. Plant Science 2003;165:1221-1227.
5. Biljana B, Stojanovic J. Chlorophyll and carotenoid content in wheat cultivars as a function of mineral nutrition. Archives of Biological Science Belgrade 2005;57(4):283-290.
6. Asada K, Takahashi M, Nagate M. Assay and inhibition of spinach superoxide dismutase. Agricultural and Biological Chemistry. 1974;38(2):471-473.
7. Ezzat-Ollah OE, Mohammad RS, Soltan AM, Hoshang A, Mahmood T. Water stress, antioxidant enzyme activity and lipid peroxidation in wheat seedling. Journal of Food and Agricultural Science 2007;5(1):149-153.
8. Foyer CH, Halliwell B. The presence of glutathione and glutathione reductase in chloroplasts. A proposed role in ascorbic acid metabolism. Planta 1976;133:21-25.
9. Goudarzi M, Pakniyat H. Salinity causes increase in proline and protein contents and peroxidise activity in wheat cultivars. Journal of Applied Science 2009;9(2):348-353.
10. Ezatolla F, Mohsen F, Shokuh D. Comparision between effective selection criteria of drought stress tolerance in bread wheat landraces of Iran. *Annals. Biol. Res.* 2012;3:3381-3389.
11. Halliwell B, Foyer CH. Properties and physiological functions of a glutathione reductase purified from spinach leaves by affinity chromatography. Planta. 1978;139:9-17.
12. Mostafa A, Mostafa V, Mohammad Z, Hossein S. Antioxidant protection and electrolyte leakage in durum wheat under drought stress condition. Journal of Applied Sciences Research, 2011;7(3):236-246.
13. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiology 1981;22(5):867-880.
14. Ranibye T, Asim K. Drought stress tolerance and antioxidant system in Citenanthe setosa. Acta Biol. Cracoviensia 2006;48:89-96.
15. Rachana D, Narinder K, Anil KG. Potentials of antioxidant enzymes in depicting drought tolerance of wheat (*Triticum aestivem* L.) Indian J. Biochem & Biphys 2011;49:257-265.
16. Sarvajeet SG, Narendra T. Reactive oxygen species and antioxidantmachinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry, 2010;480:909-930.
17. Sarika A, Sairam RK, Srivastava GC, Aruna T, Meena RC. Role of ABA, salicylic acid, calcium and hydrogen peroxide on antioxidant enzymes induction in wheat seedlings. Plant Science, 2005;169:559-570.
18. Shannon LM, Key E, Law JY. Peroxidase isoenzymes from horse reddish roots: Isolation and physical properties. Journal of Biological Chemistry 1966;241:2166-2172.
19. Sinha AK. Calorimetric assay of catalase. Analytical Biochemistry, 1972;47:389-395.
20. Zong-Bo Q, Xiao L, Xiang-Jun T, Ming Y. Effects of CO_2 laser pre-treatment on drought stress resistance in wheat. Journal of Photochemistry and Photobiology 2008;90:17-25.