J. Wheat Res. 5 (2) : 12-17

Received: 21 September 2013/ Accepted: 23 October 2013
@ Society for Advancement of Wheat Research

PGRs improve carbohydrate metabolism and yield attributes in wheat (*Triticum aestivum* L.) under water deficit condition

Sharad Kumar Dwivedi[1], Ajay Arora[*], Shiv Dhar Singh, Gyanendra Pratap Singh, Shivani Nagar and Santosh Kumar[1]

Indian Agricultural Research Institute
New Delhi-110 012, India

Abstract

Drought is the most significant factor restricting wheat production in the majority of agricultural fields not only in India but also in other parts of the world. A pot culture experiment was conducted to study the effect of exogenous application of plant growth regulators {TDZ; 0.01 µL/L + PBZ; 30 µL/L + AsA; 100 µL/L} in combination on two contrasting wheat cultivars *viz.*, HD 2733 (relatively susceptible), HD 2987 (relatively tolerant) differing on the basis of their tolerance to drought. The results revealed that water stress condition leads to the reduction in carbohydrate metabolism (starch content). Growth (leaf area and plant biomass) and yield related traits also showed significant reduction under water stress condition, while combined application of cytokinin, paclobutrazol and ascorbic acid enhanced the carbohydrate metabolism, yield and its components.

Keywords: Cytokinin, paclobutrazol, ascorbic acid, carbohydrate metabolism, yield attributes

Introduction

Water stress is a global problem, which is a severe threat for sustainable agriculture (Shao *et al.,* 2005). In developing countries 37 per cent of the area is semi arid in which available moisture is the primary constraint to wheat production. Development of adequate agricultural strategies to counteract this effect and minimize drought related yield loss could be oriented towards emphasizing the urgent need to develop adaptive agricultural strategies for a changing environment. These ranges from changes in traditional management and agronomic practices to the use of marker-assisted selection for the improvement of drought-related traits and the development of transgenic crops with enhanced tolerance of drought and improved water use efficiency that could minimize drought related losses and ensure food production for a growing population (Rivero *et al.*, 2007). Drought stress is a decrease of soil water potential so plants reduce their osmotic potential for water absorption by congestion of soluble carbohydrates and in other words osmotic regulation is performed (Martin *et al.,* 1993). Therefore osmotic regulation will help to cell development and plant growth in water stress (Pessarkli, 1999). Accumulations of soluble carbohydrates increase the resistance to drought stress in plants (Keyvan, 2010). Earlier reports mentioned that sugars protect the cells during drought by the following mechanism; the hydroxyl groups of sugars may substitute for water to maintain hydrophilic interactions in membranes and proteins during dehydration. Thus, sugars interact with proteins and membranes through hydrogen bonding, thereby preventing protein denaturation (Al-Rumaih and Al-Rumaih, 2007). Materials such as soluble carbohydrates have a role in osmotic regulations and conservation mechanism (Martin *et al.*, 1993).

[1] Current address: ICAR Research Complex for Eastern Region, Patna-800 014
[*] Corresponding author's email: *romiarora@yahoo.com, santosh9239@gmail.com*

Various plant hormones / plant growth regulators (PGRs) like ascorbic acid, paclobutrazol, cytokinin, etc. play a crucial role as a protective agent under stress and protect the crop plants from severe injury. Increasing evidence suggests that ascorbate peroxidase provides resistance to various environmental stresses in plants (Kwon *et al.*, 2002). Paclobutrazol (PBZ) is one of triazole compounds, widely used as growth retardant, induces mild stress tolerance in seedlings and adult plants. More specifically, PBZ has been reported to protect plants against drought stress (Fletcher *et al.*, 2000). Several studies have demonstrated the efficiency of this compound to reduce the negative effects of water stress (Banon *et al.*, 2001; Berova and Zlatev, 2003).

In wheat, growth is affected by water stress which can reduce the final number of tillers per plant by reduced tiller production and (or) increased tiller mortality (Fischer, I973; Jatoi *et al.*, 2011). The number of kernels per spike of wheat has been reported to be most severely reduced by water stress during the fifteen days prior to anthesis, while water stress during grain filling reduces kernel weight, yield decreases from stress at this stage are usually less severe than from stress just prior to anthesis (Musick and Dusek, 1980). Spray of vitamin C increased grain yield of wheat by influencing many physiological processes such as stimulates respiration activates, cell division and many enzymes activities as reported by Zewail (2007). Ascorbic acid foliar application increased stem and leaf dry weight and leaf fresh weight. In addition, an increase was observed in grain weight when plants were treated by ascorbic acid at two growth phases (Dolatabadian *et al.*, 2010). Paclobutrazol, a potential plant growth regulator also perform multiple task to mitigate the drought upto some extent and enhance the grain yield (Mostafa and Gaballah, 2012). Resently, combined application of PGRs (TDZ, PBZ and AsA) enhanced the plant biomass, leaf area and grain yield of wheat under water deficit stress condition (Dwivedi *et al.*, 2012).

With this background, a study was undertaken to examine the combined effect of cytokinin {thidiazuron; TDZ)} a synthetic cytokinin, paclobutrazol (PBZ) and ascorbic acid (AsA), on induced changes of carbohydrate metabolism and yield attributes of wheat under moisture deficit stress condition. Increase knowledge of the regulatory mechanisms controlling plant C economy is necessary for improving yield under adverse situation.

Materials and methods

Plant material and growth conditions: Two wheat varieties HD 2987 (relatively drought tolerant) and HD 2733 (relatively drought sensitive), were selected as suggested by breeders. Sowing was done in 30 cm earthen pots with clay loam soil and farmyard manure in 3:1 ratio during winter season and supplied with 60, 60 and 60 kg $ha^{-1}$of N, P, K, respectively, in the form of urea, single super phosphate and muriate of potash at the time of sowing. Remaining 60 kg N ha^{-1} was given after 25 days of sowing. Plants were subjected to water stress after 55 days after sowing for imposing water deficit stress during reproductive growth stage. Cytokinin, paclobutrazol and ascorbic acid treatment in combination was given by spraying Thidiazuron (TDZ) ; 0.01 µL/L, paclobutrazol (PBZ) ; 30 µL/L and ascorbic acid (AsA) ; 100 µL/L concentration at 40 DAS and 60 DAS. The plants were sampled and observations were taken for growth and biochemical parameters at pre-anthesis (67-70 DAS); anthesis (77-80 DAS) and post-anthesis stages (87-90 DAS) of the wheat plants as described above. Three replications with five pots per replication were taken for each variety. Fully expanded flag leaves were used for recording biochemical observations.

Total soluble sugars: Total sugar was determined by anthrone reagent method (McCready *et al.*, 1950). One ml of sugar sample was taken and to this 4 ml solution of anthrone regent was added. The mixture is heated on a boiling water bath for 8 min followed by cooling. The optical density of green to dark green colour was read at 630 nm in UV-visible spectrophotometer (model Specord Bio-200, AnalytikJena, Germany). A blank and two freshly prepared glucose standards were also included with each set of samples.

Estimation of starch content: Starch content was also determined by anthrone method (McCready *et al.,* 1950). Absorbance was measured at 620 nm. A reference standard curve was prepared using glucose in the range of 25-300 µg.

Growth and yield parameters: Plant biomass was calculated by the plants harvested at various growth stages and separated into stems and leaves then dried in an oven at 80 °C for four hours and then at 60 °C till constant dry weight was recorded. Dry weights were recorded and expressed as g $plant^{-1}$. Leaf area of flag leaf, main shoot leaves and total plant leaf area was measured using a standard leaf area meter (Model LiCOR 3100) and was expressed as cm^2 $plant^{-1}$. Following yield components were recorded at the time of final harvest, number of tillers; number of grains per ear, grain yield per plant, 1000-grain weight (g).The harvest index was calculated as the ratio of the economic yield to biological yield and was expressed as percentage.

Harvest index = (Economic yield / Biological Yield) X 100

Statistical analyses: The data was analysed statistically using 3 factorial CRD (Biochemical analysis and growth parameters) and 2 factorial CRD (yield attributes) design and CD at 5 per cent and ANOVA was calculated. The analysis was done using OPSTAT programme available online on CCS Agricultural University, Hisar web site.

Results and discussion

Total sugar and starch content: Total sugar (mg g^{-1}dw) was estimated to assess the carbon status of plants affected by PGRs in two cultivars differing in their tolerance to water deficit stress at reproductive stages of wheat (Fig 2). Under water deficit stress condition at pre-anthesis stage (67-70 DAS) mean total sugar was increased by 35 per cent in comparison to control wheat plants. While, water deficit plants with PGRs in combination increased the mean total sugar by 2 per cent in comparison to water deficit plants without PGRs. PGRs in combination treatment enhanced the total sugar content in both the wheat cultivars at all three developmental stages under control condition. Similar trends were observed for PGRs enhanced total sugar under water deficit stress condition at anthesis (77-80 DAS) as well as post-anthesis (87-90 DAS). Higher differences in sugar content were observed in wheat plants treated with PGRs at later developmental stage of anthesis and post-anthesis.

Starch content (mg g^{-1}dw) was estimated to assess the carbon status of plants affected by PGRs in both the cultivars differing in their tolerance to water deficit stress at reproductive stages of wheat (Fig 1). Under control condition, wheat plants mean starch content at pre-anthesis stage was (74.57), it increased at anthesis and has highest value (101.83) but reduction in starch content during post anthesis stage was observed (57.84). However, the mean starch content was declined by 62 per cent at pre-anthesis stage under water deficit stress in comparison to control wheat plants. Cultivar differences were observed more under water deficit stress as compared to the control wheat plants affected by PGRs. Higher differences in starch content were observed in wheat plants treated

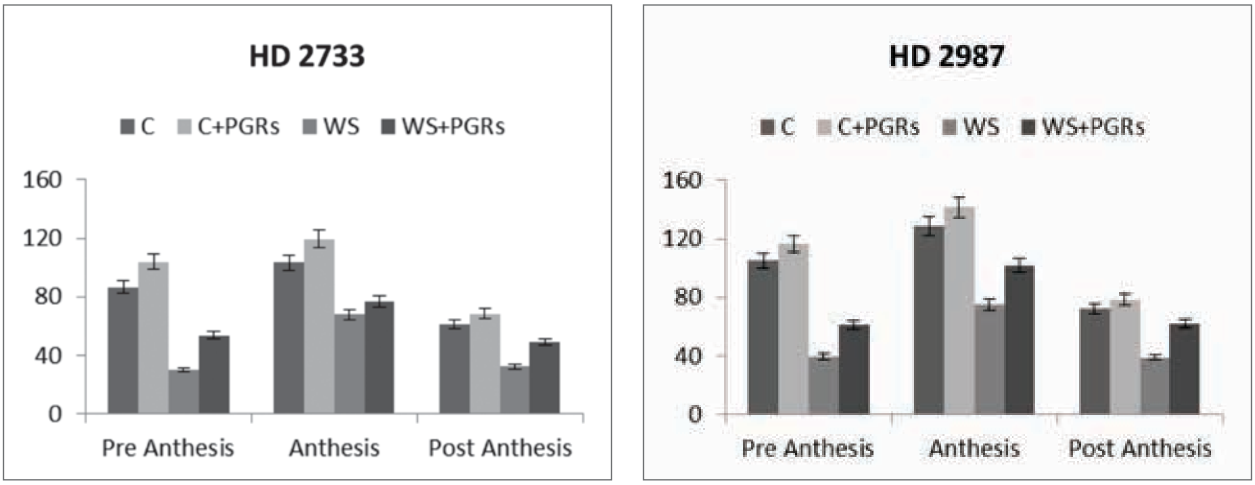

Fig 1. Combined effect of cytokinin {Thidiazuron; TDZ (0.01 µL/L)}, paclobutrazol (PBZ; 30 µL/L) and ascorbic acid (AsA; 100 µL/L) on starch content (mg g^{-1}dw) at three different growth stages in two contrasting cultivars affected by water deficit stress in wheat.

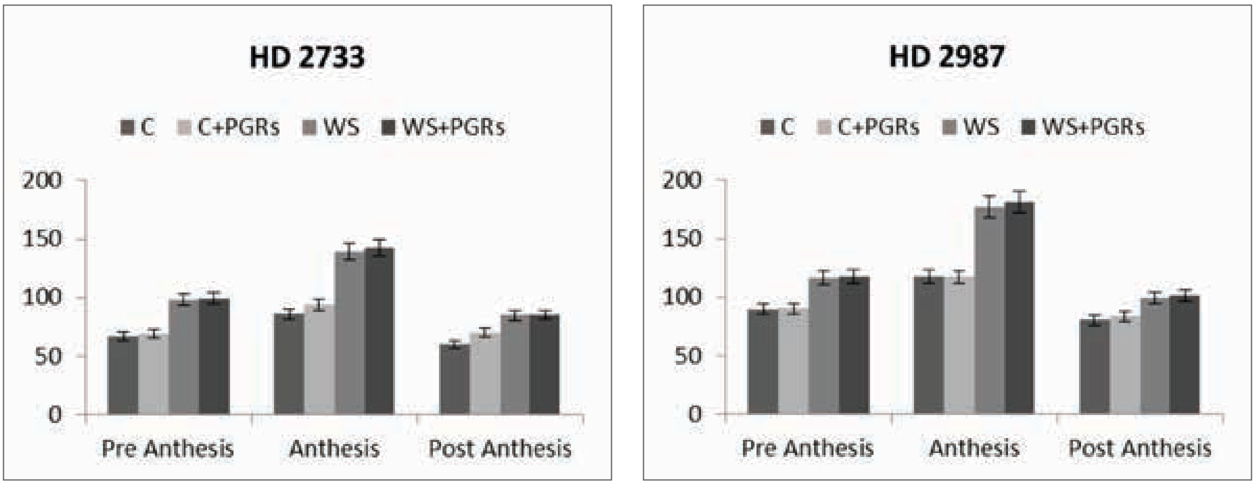

Fig 2. Combined effect of cytokinin {Thidiazuron; TDZ (0.01 µL/L)}, paclobutrazol (PBZ; 30 µL/L) and ascorbic acid (AsA; 100 µL/L) on total starch content (mg g^{-1}dw) at three different growth stages in two contrasting cultivars affected by water deficit stress in wheat.

with PGRs at later developmental stage of anthesis and post-anthesis.

The results of this study also indicated an increase in the content of total sugars and starch from pre-anthesis to anthesis, later decreased. The control recorded similar content of starch during senescence; in contrast, plants sprayed with PGRs registered a higher content of starch until 77-80 DAS, and then dropped. In *Nicotiana tabacum*, *Zea mays* and *Arabidopsis*, soluble sugars increased during natural senescence (Wingler *et al.*, 1998; Pourtau *et al.*, 2006). It is not yet clear what causes the accumulation of sugars in senescent leaves under stressed conditions despite a visible fall of photosynthetic activity (Wingler *et al.*, 2006), nor in which cellular compartments sugars accumulate (Pourtau *et al.,* 2006) similar to our results where total sugars were more in stressed plants in all the studied cultivars. The increase of sugars is attributed to the breaking of starch or the preferential export of N_2 (Wingler *et al.*, 1998). Sugars have long been known to increase in a wide range of plants grown at low moisture levels and under salinity, e.g. in wheat (Vassiliev and Vassiliev, 1936), pasture grasses (Julander, 1945) and cotton (Eaton and Ergle, 1948). The rate and extent of increase in sugar content depends on the environmental conditions, species, and even on the genotype within the same species.

Growth and yield parameters: Total biomass (g $plant^{-1}$) was measured at three different developmental stages of wheat i.e., pre-anthesis, anthesis and post-anthesis

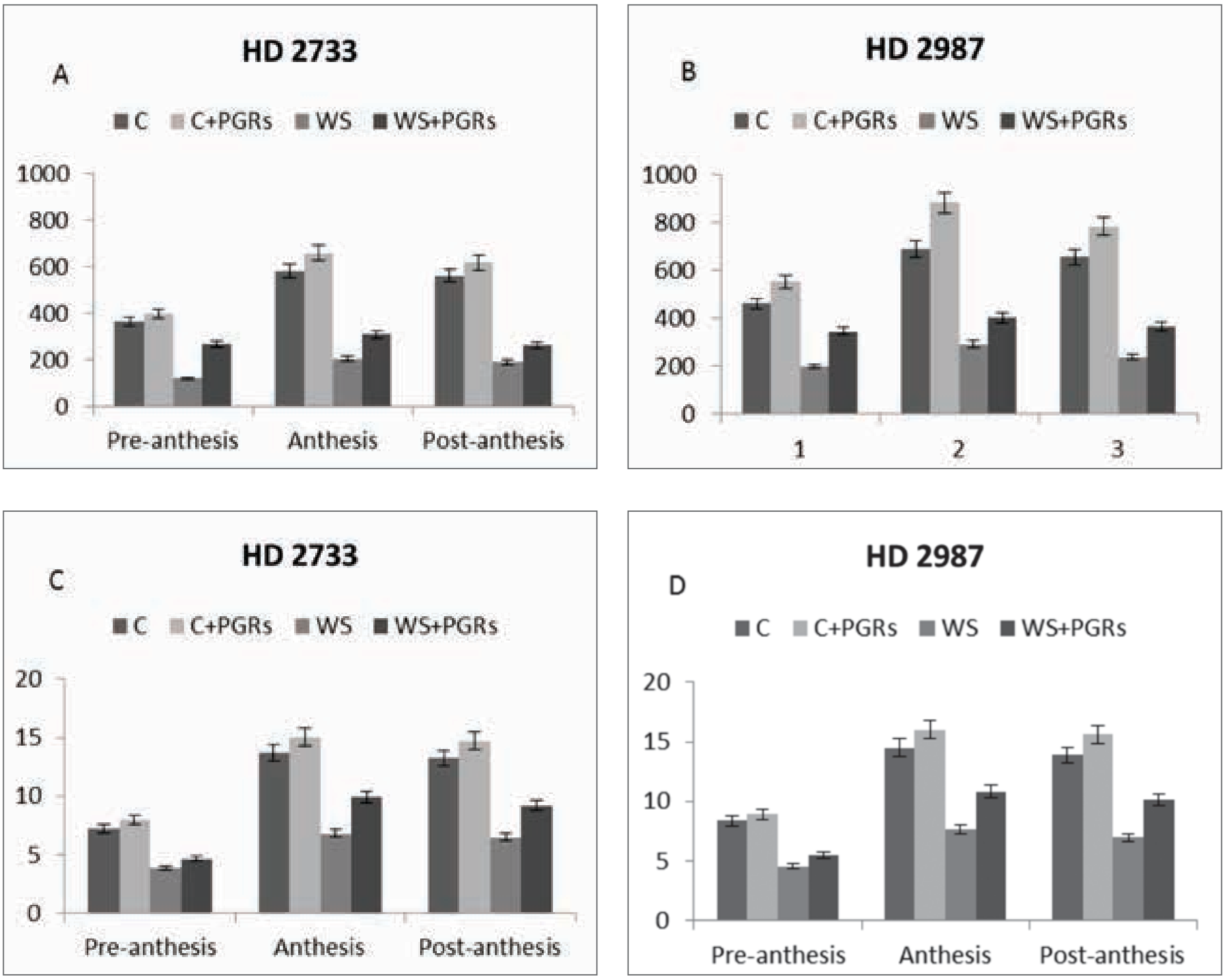

Fig. 3. Combined effect of cytokinin {Thidiazuron; TDZ (0.01 µL/L)}, paclobutrazol (PBZ; 30 µL/L) and ascorbic acid (AsA; 100 µL/L) on total leaf area (cm^2 $plant^{-1}$) (A & B) and total biomass (gm $plant^{-1}$) (C & D) at three different growth stages in two contrasting cultivars affected by water deficit stress in wheat.

(Fig. 3). Cultivar differences on total biomass were observed more under water deficit stress as compared to the control wheat plants affected by cytokinin, paclobutrazol and ascorbic acid treatment in combination. Control wheat plants of cultivar HD 2987 showed enhancement of total biomass by 6 per cent treated by cytokinin, paclobutrazol and ascorbic acid treatment in combination as compared to 18 per cent in water deficit plants treated with same PGRs at pre-anthesis stage. While, in HD 2733 it was 9 and 18 per cent, respectively. The sensitive cultivar, HD 2733 was more responsive to cytokinin, paclobutrazol and ascorbic acid treatment in combination treatment as compared to tolerant cultivar, HD 2987 under water stress condition. Leaf area (cm^2 $plant^{-1}$) was measured at three different developmental stages of wheat (Fig. 3). Cultivar differences on leaf area were observed more under water deficit stress as compared to the control wheat plants affected by cytokinin, paclobutrazol and ascorbic acid treatment in combination. Control wheat plants of cultivar HD 2987 showed enhancement of leaf area of around 20 per cent treated by cytokinin, paclobutrazol and ascorbic acid treatment in combination as compared to 74.8 per cent in water deficit plants with cytokinin, paclobutrazol and ascorbic acid treatment in combination at pre-anthesis stage. While, in HD 2733 it was 9 and 122 per cent, respectively.

Grain yield per plant (gm) were also recorded at the time of harvest in wheat plants (Table 1). There was 4% increased in grain yield per plant in treated control plants while it was 7 per cent increased in water deficit condition with cytokinin, paclobutrazol and ascorbic acid treatment in combination in tolerant HD 2987 cultivar of wheat. Similarly, in HD 2733 it was 13 and 4 per cent, respectively. Grain numbers

Table 1. Combined effect of cytokinin {Thidaizuron; TDZ (0.01 µL/L)}, paclobutrazol (PBZ; 30µL/L) and ascorbic acid (AsA; 100µL/L) on yield and yield attributes at harvest in two contrasting cultivars affected by water deficit stress in wheat.

Treatment	Control (C)			C+ (TDZ+PBZ+AsA)			Water stress (WS)			WS + (TDZ+PBZ+AsA)		
	HD 2733	HD 2987	Mean	HD 2733	HD 2987	Mean	HD 2733	HD 2987	Mean	HD 2733	HD 2987	Mean
Grain No/ Ear	32.00	34.67	33.33	38.67	35.00	36.83	28.00	30.00	29.00	36.00	37.33	36.66
Grain yield/ Plant	09.33	12.00	10.66	10.67	12.50	11.58	8.33	09.17	8.75	8.67	9.83	9.25
1000 grain weight	46.29	53.38	49.83	49.64	57.97	53.80	40.27	46.97	43.62	42.97	48.25	45.61
Harvest index	35.28	40.18	37.73	45.23	40.97	43.10	32.61	36.67	34.64	33.54	38.45	36.04

per panicle were also recorded at the time of harvest in wheat plants. There was 1 per cent increased in grain number per panicle in treated control plants while there was 24 per cent increased in water deficit condition with cytokinin, paclobutrazol and ascorbic acid treatment in combination in tolerant HD 2987 cultivar of wheat. While, in HD 2733 it was 21 and 29 per cent, respectively. 1000 grain weight (g) was also recorded at the time of harvest in wheat plants (Table 1). There was 8 per cent increased in 1000 grain weight in treated control plants while it was 3 per cent increase in water deficit condition with cytokinin, paclobutrazol and ascorbic acid treatment in combination in tolerant (HD 2987) cultivar of wheat. Similarly, in HD 2733 it was 7 and 6 per cent, respectively. Harvest index (HI) was also recorded to assess the partitioning efficiency of wheat plants at the time of harvest (Table 1). There was 2 per cent increase in HI in treated control plants while it was 5 per cent increase in water deficit condition with cytokinin, paclobutrazol and ascorbic acid treatment in combination in tolerant HD 2987 cultivar of wheat. While, in HD 2733 it was 28 and 3 per cent, respectively.

In the present study the results showed significant improvement in grain yield and harvest index of both the cultivars treated with cytokinins, paclobutrazol and ascorbic acid in combination in control as well as stressed plants. This reflected towards the enhancement of total biomass, spike number and leaf area of the treated plants. Similar kind of observations were made (Shah and Paulsen, 2011) in cytokinin treated black cumin plants where they observed appreciable improvement in the yield parameters. Different concentration of paclobutrazol could increase the tiller number and root-shoot ratio of *F.arundinacea* and treatment with higher concentration of paclobutrazol had extremely significant difference with CK. Recently, Mostafa and Gaballah (2012) reported that barley yield significantly improves with the application of PBZ under water deficit stress condition.

In conclusion, under drought relatively tolerant cultivar (HD 2987) maintained higher content of sugar and starch. The PGRs application improves the carbohydrate metabolism in terms of sugar and starch content thus protects the plant from severe injury due to water deficit stress. Growth and yield and yield attributes shows positive response to PGRs under water deficit stress condition. In nut shell PGRs helps in mitigation of water stress and reduced the percent reduction in yield due to water stress.

Acknowledgement

Financial assistance provided by CSIR is gratefully acknowledged.

References

1. Al-Rumaih MM and Al-Rumaih MM (2007). Physiological response of two species of datura to uniconazole and salt stress. *Journal of Food, Agriculture and Environemt* **5**:450-453.
2. Banon S, Ochoa J, Fernandez JA and Franco JA (2001). Paclobutrazol as aids for *Phillyrea angustifolia*. *Horticulture Science* **36**:449-500.
3. Berova M and Zlatev Z (2003). Physiological response of paclobutrazol-treated triticale plants to water stress. *Plant Biology* **46**:133-136.
4. Dolatabadian A, Modarressanavy SAM and Asilan KS (2010). Effect of ascorbic acid foliar application on yield, yield components and several morphological traits of grain corn under water deficit stress condition. *Notulae Scientia Bio*logy **2**:45-50.
5. Dwivedi SK, Singh VP, Singh GP and Arora A (2012). Combined effect of cytokinin, paclobutrazol and ascorbic acid on nitrogen metabolism and yield of wheat (*Triticum aestivum* L.) under water deficit stress condition. *Indian Journal of Plant Physiology* **17**:259-267.
6. Eaton FM and Ergle DR (1948). Carbohydrate accumulation in the cotton plant at low moisture levels. *Plant Physiology* **23:**169-187.
7. Fischer RA (1973). Developmental morphology of the inflorescene in hexaploid wheat cultivars with and without Norin 10 in their ancestry. *Canadian Journal of Plant Science* **53:**715.
8. Fletcher RA, Gilley A, Sankhla N and Davis TV (2000). Triazoles as plant growth regulators and stress protectants. *Horticultural Reviews* **24:**55-138.
9. Jatoi WA, Baloch MJ, Kumbhar MB, Khan NU and Kerio MI (2011). Effect of water stress on physiological and yield parameters at anthesis stage in elite spring wheat cultivation. *Sarhad Journal of Agriculture* **27**:59-65.
10. Julander O (1945). Drought resistance in range and pasture grasses. *Plant Physiology* **2** :573-599.
11. Keyvan S (2010). The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars. *Journal of Animal and Plant Sciences* **8**:1051-1060.
12. Kwon SY, Jeong YJ, Lee HS, Kim JS, Cho KY and Allen R (2002). Enhanced tolerances of transgenic tobacco plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against methyl viologen mediated oxidative stress. *Plant Cell Environment* **25:**873-882.
13. Martin M, Micell F, Morgan JA, Scalet M and Zerbi G (1993). Synthesis of osmotically active substances in winter wheat leaves as related to drought resistance of different genotypes. *Journal of Agronomy and Crop Science* **171**:176-184.
14. McCready RM, Guggloz J, Silviera V and Ovens (1950). Determination of starch and amylase in vegetables. *Analytical Chemistry* **22**:1156-1158.
15. Mostafa MR and Gaballah MS (2012). Improving barley yield grown under water stress conditions. *Research Journal of Recent Sciences* **1**:1-6.
16. Musick JK and Dusek DA (1980). Planting date and water deicit effects on development and yield of irrigated winter wheat. *Agronomy Journal* **72:**45-52.
17. Pessarkli M (1999). Hand book of plant and crop stress. Marcel Dekker Inc. New York, pp.1254.
18. Pourtau N, Jennings R, Pelzer E, Pallas J and Wingler A (2006). Effect of sugar-induced senescence on gene expression and implications for the regulation of senescence in *Arabidopsis. Planta* **224**:556-568.
19. Rivero RM, Kojima M, Gepstein A, Sakakibara H, Mittler R, Gepstein S and Blumwald E (2007). Delayed leaf senescence induces extreme drought tolerance in a flowering plant. *Proceedings of National Academy of Science* USA **104**:19631-19636.
20. Shah NH and Paulsen GM (2011). Intraction of drought and high temperature on photosynthesis and grain filling of wheat. *Plant Soil* **257:**219-226.
21. Shao HB, Liang ZS and Shao M (2005). Changes of some anti-oxidative enzymes under soil water deficits among 10 wheat genotypes at maturation stage. *Colloids Surf. B: Biointerfaces* **45**:7-13.
22. Vassiliev IM and Vassiliev MG (1936). Changes in carbohydrate content of wheat plant during the process of hardening for drought resistance. *Plant Physiology* **11:**115-125.
23. Wingler A, Purdy S, MacLean JA and Pourtau N (2006). The role of sugar in integrating environmental signals during the regulation of leaf senescence. *Journal of Experimental Botany* **57**:391-399.
24. Wingler A, Von Schaewen A, Leegood RC, Lea PJ and Quick WP (1998). Regulation of leaf senescence by cytokinin, sugar, and light. Effects on NADH-depend hydroxypyruvatereductase. *Plant Physiology* **116**:329-335.
25. Zewail YMR (2007). Improvement of wheat productivity by using some biofertilizers and antioxdants. *M.Sc. Thesis, Fac. Agri*culture Moshtohor, Banha University.