Paclobutrazol-induced alleviation of water-deficit damage in relation to photosynthetic characteristics and expression of stress markers in contrasting wheat genotypes

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Abstract

Our experiment was conducted in order to find out effects of paclobutrazol (PBZ; 30 µl l^{-1}) on morphology, photosynthetic process, and stress markers under water surplus and deficit conditions in several wheat genotypes. Study revealed that relative water content (RWC), photosynthetic rate, and maximal quantum yield of PSII (F_V/F_M) was improved after a PBZ application both under irrigation and water deficit across the genotypes, while the stomatal conductance was reduced. Further, the application of PBZ led to reduced leaf area in wheat genotypes. Moreover, a proline content was higher in the wheat genotypes under water stress as compared to the irrigated plants. The application of PBZ led to downregulation of the proline content under water deficit, while there was no significant change in the content and activity under irrigation with or without the PBZ treatment. These findings indicated that due to the application of PBZ the wheat genotypes might sense a lower stress level (indicated by the proline content) and better drought tolerance (according to RWC and photosynthetic characteristics).

Additional key words: leaf area; photosynthesis; proline.

Introduction

Drought is an increasing threat all over the world. Drought stress can reduce the grain yield up to 17–70% (Shao *et al*. 2005). Climate models have indicated that drought episodes become more frequent because of the long-term effects of global warming (IPCC 2007). Among the physiological processes, photosynthesis is the basic determinant of plant growth and productivity and an ability to maintain the rate of carbon assimilation under environmental stress is of fundamental importance to plant production (Lawlor 1995). Wheat genotypes vary in the timing of senescence initiation and also in the subsequent rate of leaf senescence. Thus, delaying leaf senescence has become a desirable trait for agronomy (Subhan and Murthy 2001). Flag leaf photosynthesis in wheat contributes to about 30-50% of assimilates for grain filling. Since the initiation of grain filling coincides with the onset of senescence, flag leaf photosynthesis is one of the most important basis for determining the grain yield.

Plant growth regulators are believed to play major roles in regulating senescence (Dwivedi *et al*. 2012). Paclobutrazol, a derivative of triazole group, is widely used for protection against water stress (Fletcher *et al*. 2000). The principal mode of PBZ action is through an inhibition of gibberellin biosynthesis (Dalziel and Lawrence 1984). Triazoles are generally more effective in retarding growth when applied to soil or directly to stems as compared to foliar applications (Davis *et al*. 1988). PBZ is readily taken up through the roots, stems, and leaves, but is transported almost exclusively in the xylem to its site of action, the subapical meristem, where it has a persistent effect (Anon 1984). PBZ-induced drought tolerance has been associated with a decrease in transpiration, plant height, biomass, and leaf area and an increase in stomatal resistance. Plant protection from apparently unrelated stress by triazoles is mediated by a reduction of free-radical damage and increase in an antioxidant potential (Jaleel *et al*. 2007). In most crop species, typical leaf RWC is about 60–70% at the beginning of wilting. PBZ application maintain

water turgidity in plant leaves during drought stress (Jungklang *et al.* 2015). PBZ had the ability to maintain a leaf water content for 30–40 d of water-deficit stress (Jungklang and Saengnil 2012). Biochemical effects of the triazole include detoxification of active oxygen species, increased contents of antioxidants and chlorophyll (Chl) (Raddy and Gaballah 2012). Photosynthetic rate was improved by uniconazole, which contributed to the better yield, regardless of water conditions (Qiu *et al.* 2005). It is also shown that the increase in photosynthetic pigments due to application of PBZ might be attributed to higher stimulation of stomatal regulation (Navarro *et al.* 2007). Increasing PBZ concentrations decreased a plant height, plant diameter, and plant dry mass, but increased leaf Chl content in both experiments (Grossi *et al.* 2005). Proline is well known as an osmotic regulator that can reduce osmotic damage (Slama *et al.* 2008; Surender Reddy *et al.* 2015). However, Tatar and Gevrek (2008) pointed out that proline appeared to be mainly involved in a protection against oxidative stress rather than an osmotic adjustment during initial steps of water stress in wheat. Under normal conditions,

proline is shown to be involved in plant development, while under biotic and abiotic stresses it conferred resistance to plant. One of the two main enzymes in the proline biosynthesis pathway, Δ1-pyrroline-5-carboxylate synthetase (P5CS), has been demonstrated to play a significant role in proline accumulation in plants under water stress (Amini *et al.* 2015). Poustini *et al.* (2007) have shown that in wheat cultivars a larger concentration of proline was accumulated in salt-sensitive rather than salt-tolerant cultivars under salinity stress. Jungklang and Saengnil (2012) reported that proline accumulation was lower in the PBZ-treated *Curcuma* compared with untreated plants.

Therefore, the aim of present study was to find out effect of PBZ (30 µl l^{-1}) on wheat genotypes for improvement of their drought tolerance in terms of water status, photosynthetic characteristics, and a stress marker (proline). A better understanding of the PBZ effects, which enable wheat plants to adapt to drought stress and maintain their physiological activity during stress periods, would help in future research.

Materials and methods

Plant material: Four wheat genotypes, such as HD2987, C306 (relatively drought tolerant), HD2888, and HD2733 (relatively drought sensitive) were obtained from Division of Genetics and Plant Breeding, Indian Agricultural Research Institute, New Delhi, India. Sowing was done in 30-cm earthen pots with clay loam soil and farmyard manure in 3:1 ratio during the winter season and supplied with 60, 60, and 60 kg ha^{-1} of N, P, and K, respectively, in the form of urea, single superphosphate, and muriate of potash at the time of sowing. Remaining 60 kg(N) ha^{-1} was given 25 d after sowing (DAS), in the form of urea. Drought stress conditions were imposed to plants during a reproductive stage after 55 DAS. The treatment with PBZ (30 µl l^{-1}) was applied 62 DAS as foliar spray, one week after withholding of irrigation, based on tip drying and leaf curling. A concentration of the plant growth regulators (PBZ) was selected according to our preliminary results (data not shown) and literature available (Dwivedi *et al.* 2012).

Plant sampling: The plants were sampled and observations were taken for growth and physiological characteristics at pre-anthesis (67–70 DAS), anthesis (77–80 DAS), and post-anthesis (87–90 DAS) stages. For each treatment, five pots in three replications were maintained in order to record the physiological observations. The uppermost fully expanded flag leaf was used for recording the observations.

Relative water content (RWC): Leaf RWC was estimated by recording the turgid mass (TM) from 0.5 g of fresh leaf samples (FM) after 4 h in water, followed by

drying in hot air oven till constant mass (dry mass, DM) was achieved (Weatherley 1950). It was calculated as:

$$RWC = [(FM - DM)/(TM - DM)] + 100$$

Leaf area (LA) was determined by a leaf area meter (*LAI-3000A*, *LICOR*, USA).

Total Chl content was estimated spectrophotometrically (*UV-Vis* spectrophotometer, *Thermo*, USA) in Chl extracts prepared by incubating leaf tissues in dimethyl sulfoxide (DMSO). DMSO renders plasmalemma permeable thereby, causing the leaching of the pigments (Hiscox and Israelstam 1979). The Chl content was expressed in mg g^{-1}(DM) (Arnon 1949).

$$\text{Total Chl} = (20.2\ OD_{645} + 8.02\ OD_{663})\ V/1000\ w$$

where OD_{663} = absorbance values at 663 nm; OD_{645} = absorbance values at 645 nm; w = mass of the sample in mg; V = volume of the solvent used (ml).

Net photosynthetic rate (P_N) and stomatal conductance (g_s) were measured on fully expanded flag leaves using portable infrared gas analyzer (*LI-6400 Model, LICOR,* USA). P_N was determined at different growth stages between 10:00 and 11:30 h by providing artificial light source with light intensity of 1,000 µmol(photon) m^{-2} s^{-1}. P_N was expressed as µmol(CO$_2$) m^{-2} s^{-1}, while g_s was expressed as mol(H$_2$O) m^{-2} s^{-1}.

Photochemical efficiency (F_V/F_M) was also measured on

fully expanded leaves of five different plants using *LICOR 6400* (*LICOR,* USA). The leaves were covered by leaf clips (black) for 20-min dark adaptation. Then the photochemical efficiency was recorded and it was expressed as F_V/F_M ratio (Brestic and Zivcak 2013). Here, F_V is variable fluorescence and F_M is maximum fluorescence.

Proline was estimated according to Bates *et al*. (1973) using *UV-Vis* spectrophotometer (*Thermo*, USA). Leaf segments (0.5 g) from each replication of each genotype

were taken for the proline estimation. The proline content was determined from a standard curve and calculated on a fresh mass basis as μmol g^{-1}(FM).

Expression of water-stress marker gene for pyrroline-5-carboxylate synthase: For RT-PCR expression analysis and cloning of cDNAs, following oligonucleotide primers were designed manually, and oligo quality (in order to avoid primer dimer, self dimer, *etc*.), guanine cytosine percentage (GC%) and T_m were analyzed by using *Oligo analyzer 3.0* tool (*Integrated DNA Technologies*, USA).

Name	Sequence	Length (bases)	GC %	Tm (ºC)	Product size (bp)
WP5CS F	CCCGGCGGCCACAAGAA	17	71	53.5	689
WP5CS R	GCCCAGCAAATAAACCAATACA	21	43	53.5	

Isolation of total RNA: Total RNA was extracted from flag leaf tissues of plants using *RNA Easy* kit (*Qiagen Inc*.) according to the manufacturer's instruction.

RT-PCR expression analysis of target genes:

Reverse transcriptase-polymerase chain reaction (RT-PCR) mixture using *Qiagen One Step RT PCR* kit with gene specific forward and reverse primers was prepared according to the protocol given (*Qiagen*, Germany) below:

S. No.	Reagents	Quantity for 50 μl of reaction mixture
1	Nuclease free water	21.0 μl
2	5X RT buffer (Tris Cl, KCl, $(NH_4)_2SO_4$, 13.5 mM $MgCI_2$, DTT; pH 8.7 at 20 ºC)	10.0 μl
3	dNTP Mix (10 mM each)	2.0 μl
4	Forward + Reverse primers (10 μM)	5.0 μl
5	RNA template (1 μg)	10.0 μl
6	RT Enzyme mix	2.0 μl
7	Total volume	50.0 μl

The reaction mixture was prepared in 0.2-ml PCR tubes and reactions were carried out using *QB 96* thermal cycler (*Quanta Biotech,* England), under the following conditions. Number of cycles was standardized as 25-27 cycles by conducting semiquantitative RT-PCR, amplified products were not visible in susceptible cultivars, when the number of cycles was less than 25. Reverse transcription: 30 min 50ºC, initial PCR activation step: 15 min 95ºC, 3-step cycling, denaturation: 1 min 94ºC, annealing: 1 min 57ºC, extension: 2 min 72ºC, final extension: 10 min 72ºC, number of cycles: 27. Linear amplification for semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) was obtained with 27 cycles. To check the amplification, an aliquot of 5 μl of the reaction mixture was run on a 0.8 % (w/v) agarose gel stained with 0.5 μg ml^{-1} ethidium bromide. 1 kb DNA ladder was included as a marker for size comparison of the amplified products. 1X

(working concentration) TAE buffer was used to prepare the gel as well as for running buffer and electrophoresis was carried out at 5 V cm^{-1}. 6X loading dye was used to load the samples. The stained DNA products were photographed using gel documentation system (*Biometra UV doc*, France).

Statistical analyses: The data wer analyzed statistically with *OPSTAT* software available online on *CCS, HISAR* web site for analysis of variance (*ANOVA*) using 3 factorial complete randomized designs for physiological parameters. Treatments were compared by computing the "*F*" test. The standard error of mean (SE) and differences between treatments were compared pair-wise by critical difference (CD) at 5% level ($p = 0.05$) of probability by using DMR test, when *F*-values were significant (Table 1S, *supplement available online*).

Results and discussion

Understanding of physiological mechanisms that enable plants to adapt to water deficit stress and maintain growth during stress period could help in screening and selection of tolerant genotypes and using this trait in breeding

programs (Zaharieva *et al*. 2001). Various observation from this experiment showed that the application of PGR as a foliar spray enhanced tolerance to drought in four wheat genotypes.

RWC was assessed in order to know the water status of the wheat genotypes. The mean RWC showed a declining trend from the pre-anthesis to post-anthesis stage (Table 1). Under water stress, the reduction in RWC was the highest (21.9%) in the genotype HD2733, followed by HD2888 (19.4%) as compared with controls at the pre-anthesis stage. The lowest reduction in RWC (14.9%) was observed in the genotype C306 at the pre-anthesis stage. The reduction in RWC under water stress was also observed in a similar pattern at the anthesis and post-anthesis stage. Moreover, due to application of PBZ the reduction in RWC was lesser in all genotypes. The genotype HD2733 was more responsive toward the PBZ application as compared to other genotypes. Due to application of PBZ, RWC was improved by 7.8% in HD2733, followed by 6.9% in HD2888 as compared to water-stress conditions at the pre-anthesis stage. This was in agreement with the findings of Jungklang and Saengnil (2012) and Jungklang *et al.* (2015) who reported that PBZ kept RWC higher than that of untreated plants when subjected to water stress for 30 d.

LA declined significantly due to water stress at all developmental stages studied (Table 2). The reduction in mean LA was higher (65.5%) in the genotype HD2733, followed by HD2888 (49.2%), while the lowest reduction (40.9%) was observed in the genotype C306 as compared with controls. Moreover, the reduction in LA was also observed after the PBZ application, although the reduction was lesser as compared to water-stress condition. LA of the genotype HD2733, followed by HD2987, was more responsive toward the PBZ application as compared to other wheat genotypes. Similar results were reported by Fernández *et al.* (2006). Chaturvedi *et al.* (2009) reported that PBZ application was accompanied by a reduction in LA.

Total Chl content: The well-known phenomenon is a loss of Chl in senescing leaves. In this study, the Chl content was higher in C306 and HD2987 (relatively tolerant genotypes) than that in HD2888 and HD2733 (relatively susceptible genotypes) during the flag leaf senescence, showing that process of leaf senescence in C306 and HD2987 was delayed (Fig. 1). The PBZ application delayed leaf senescence in all four genotypes studied. Under water-stress conditions, the reduction in the mean Chl content was the highest in the genotype HD2733 (25.5%), followed by C306 (18.9%), while the lowest reduction (18.3%) was observed in the genotype HD2987 as compared with the control at the pre-anthesis stage. Moreover, the application of PBZ had a positive impact on the Chl content under water stress. The enhancement in the content of Chl due to the PBZ application was the highest (43.4%) in the genotype HD2733, followed by HD2987 (13.5%) as compared to other two genotypes. However, higher differences in the Chl content were observed in wheat plants treated with PBZ at the later developmental stage of anthesis and post-anthesis. The present study

revealed that exogenous application of PBZ *via* foliar application helped plants in maintaining Chl and hence mitigated the adverse effects of drought stress. These findings were in line with some earlier reports in okra (Amin *et al.* 2009), and in wheat (Azzedine 2011). Grossi *et al.* (2005) also reported that PBZ-treated plants exhibited greener leaves and higher Chl content as compared with untreated plants.

Photosynthetic characteristics: P_N was estimated in order to assess the CO_2 assimilation efficiency under different treatments at three different developmental stages of wheat (Fig. 1). Our results showed that P_N of all wheat genotypes was reduced due to water stress. The reduction in mean P_N was maximum (32.4%) in the genotype HD2733, followed by HD2888 (29.2%), while the reduction was the lowest (20%) in the genotype HD2987 as compared with controls at the pre-anthesis stage. The application of PBZ on water stress-treated plants led to the enhancement in P_N by 7.9 and 13.5%, in C306 and HD2987, respectively, at the pre-anthesis stage. While, in HD2888, the enhancement was 26.7% and it was 30.7% in the sensitive genotype HD2733. However, under water stress and after PBZ treatment, the higher differences in P_N were observed at the later developmental stages, such as anthesis and post-anthesis stage.

Stomatal conductance (g_s) was also measured at three different developmental stages in wheat (Fig. 2). The PBZ-treated plants showed a lesser reduction in g_s in comparison to the water-deficient plants. Similar trends were observed at the anthesis as well as post-anthesis stages. Under water stress, the reduction in g_s was the lowest (51%) in the genotype C306, while the reduction was the highest (59%) in HD2888 as compared with controls at the pre-anthesis stage. The reduction in g_s was lesser due to the PBZ application in comparison to compared to water-stress conditions. The genotype HD2888, followed by HD2733, was more responsive toward the PBZ application than other two wheat genotypes. Berova and Zlatev (2003) also reported that PBZ-treated plants showed lesser suppression of photosynthetic activity with lower g_s under water stress. These finding indicated that the stomatal factors for limiting photosynthesis were overcome due to the application of PBZ.

Maximum photochemical efficiency of PSII (F_V/F_M) was also measured at three different developmental stages in wheat (Fig. 2). It was observed that water-deficient plants treated with PBZ showed an increase in their F_V/F_M (by 2.8%) in comparison to the water-deficient plants without PBZ. Under water deficit conditions, the reduction in the F_V/F_M ratio was the highest in the genotype HD2987, followed by HD2888. Due to the PBZ application, the genotypes C306 and HD2987 showed the enhancement in the F_V/F_M ratio by 0.8 and 4.6%, respectively, at the pre-anthesis stage, while, the enhancement was 4.1% in HD2733. However, higher differences in F_V/F_M were

Table 1. Effect of paclobutrazol on RWC at three different growth stages of four wheat genotypes (V1 –HD2987, V2 – C306, V3 – HD 2888, and V4 – HD 2733), under water-deficit stress (mean ± SD). PBZ – paclobutrazol; RWC – relative water content; C – control; WS – water stress. *Different letters* indicate significant differences of RWC between treatments in the same year at $p<0.05$.

Stages	Preanthesis (67–70 DAS) RWC [%]				Anthesis (77–80 DAS)				Post anthesis (87–90 DAS)			
Treatment	V1	V2	V3	V4	V1	V2	V3	V4	V1	V2	V3	V4
C	89.3 ± 1.8[a]	85.5 ± 1.3[b]	86.7 ± 1.6[b]	83.2 ± 3.3[b]	89.3 ± 0.5[a]	87.6 ± 0.3[a]	88.6 ± 1.2[a]	84.9 ± 4.3[a]	83.7 ± 1.1[a]	81.9 ± 1.5[a]	81.1 ± 0.7[a]	78.9 ± 3.5[b]
C + PBZ	90.8 ± 2.1[a]	88.2 ± 1.1[a]	88.9 ± 1.8[a]	84.4 ± 3.9[a]	91.7 ± 1.4[a]	89.2 ± 0.9[a]	89.8 ± 0.9[a]	85.6 ± 2.8[a]	84.5 ± 0.3[a]	82.7 ± 0.3[a]	81.9 ± 0.6[a]	80.9 ± 2.9[a]
WS	74.2 ± 4.0[c]	72.7 ± 2.2[d]	69.9 ± 0.8[d]	64.9 ± 0.9[d]	74.7 ± 0.4[c]	72.1 ± 2.4[c]	72.9 ± 0.7[d]	64.7 ± 0.5[c]	70.0 ± 0.3[c]	66.5 ± 0.9[c]	65.7 ± 0.4[c]	60.1 ± 0.4[d]
WS + PBZ	78.4 ± 3.8[b]	76.6 ± 0.9[c]	74.7 ± 1.8[c]	71.4 ± 1.9[c]	79.1 ± 0.9[b]	77.2 ± 1.1[b]	76.8 ± 0.7[b]	73.7 ± 0.8[b]	74.7 ± 1.7[b]	74.0 ± 1.3[b]	73.5 ± 1.8[b]	64.7 ± 0.6[c]

Table 2. Application of paclobutrazol on leaf area at three different growth stages of four wheat genotypes (V1 – HD2987, V2 – C306, V3 – HD 2888, and V4 – HD 2733), under water-deficit stress (mean ± SD). PBZ – paclobutrazol; C – control; WS – water stress. *Different letters* indicate significant differences of leaf area between treatments in the same year at $p<0.05$.

Stages	Preanthesis (67–70 DAS) LA [cm² per plant]				Anthesis (77–80 DAS)				Post anthesis (87–90 DAS)			
Treatment	V1	V2	V3	V4	V1	V2	V3	V4	V1	V2	V3	V4
C	448.3 ± 3.3[a]	429.1 ± 8.1[a]	434.3 ± 7.1[a]	351.3 ± 18.2[a]	591.4 ± 5.3[a]	561.7 ± 2.8[a]	547.5 ± 9.4[a]	473.3 ± 24.7[a]	595.9 ± 4.6[a]	563.1 ± 3.3[a]	549.3 ± 8.8[a]	473.5 ± 23.9[a]
C + PBZ	361.1 ± 5.4[b]	342.1 ± 5.9[b]	369.6 ± 18.7	275.6 ± 29.1[b]	449.9 ± 28.6[b]	474.4 ± 3.1[b]	434.6 ± 20.7[b]	396.5 ± 8.9 [b]	454.5 ± 5.0[b]	478.9 ± 2.0[b]	437.1 ± 13.5[b]	312.2 ± 7.5[b]
WS	263.3 ± 5.0[c]	253.2 ± 8.7[c]	220.8 ± 9.2[c]	121.1 ± 6.0[c]	313.5 ± 22.8[c]	276.5 ± 1.9[c]	252.7 ± 5.5[c]	190.2 ± 13.9[c]	328.4 ± 25.7[c]	282.5 ± 3.5[c]	257.9 ± 16.9[c]	192.5 ± 5.6[c]
WS + PBZ	243.4 ± 4.1[d]	242.3 ± 11.7[c]	208.0 ± 0.2[c]	108.4 ± 7.6[c]	307.3 ± 42.3[c]	250.1 ± 4.8[d]	236.7 ± 4.0[d]	178.2 ± 20.1[c]	314.9 ± 32.4[c]	270.8 ± 7.8[c]	238.9 ± 10.6[c]	178.9 ± 10.9[c]

Table 3. Effect of paclobutrazol on proline content at three different growth stages of four wheat genotypes (V1 – HD2987, V2 – C306, V3 – HD 2888, and V4 – HD 2733), under water-deficit stress (mean ± SD). PBZ – paclobutrazol; C – control; WS – water stress, FM – fresh mass. *Different letters* indicate significant differences of proline content between treatments in the same year at $p<0.05$.

Stages	Preanthesis (67–70 DAS) Proline [µmol g^{-1}(FM)]				Anthesis (77–80 DAS)				Post anthesis (87–90 DAS)			
Treatment	V1	V2	V3	V4	V1	V2	V3	V4	V1	V2	V3	V4
C	2.0 ± 0.21[c]	2.8 ± 0.12[c]	1.7 ± 0.25	1.4 ± 0.21[c]	2.5 ± 0.24[c]	3.0 ± 0.22[d]	2.0 ± 0.19[d]	1.4 ± 0.34[c]	2.8 ± 0.17[c]	3.5 ± 0.13[d]	2.4 ± 0.25[c]	1.7 ± 0.23[c]
C + PBZ	2.1 ± 0.31[c]	3.1 ± 0.14[c]	1.8 ± 0.12	1.5 ± 0.18[c]	3.0 ± 0.39[c]	3.6 ± 0.16[c]	2.2 ± 0.15[c]	1.8 ± 0.23[c]	3.4 ± 0.26[c]	4.2 ± 0.18[c]	2.7 ± 0.14[c]	2.3 ± 0.20[c]
WS	23.1 ± 2.69	24.0 ± 0.51[a]	16.8 ± 0.50[a]	18.3 ± 2.08[a]	23.3 ± 2.18[a]	25.6 ± 2.63[a]	19.7 ± 1.80[a]	20.0 ± 2.27[a]	27.1 ± 2.35[a]	29.5 ± 2.36[a]	22.3 ± 0.32[a]	21.3 ± 1.78[a]
WS + PBZ	9.3 ± 0.09[b]	12.8 ± 0.25[b]	8.1 ± 0.22[b]	4.6 ± 0.32[b]	9.1 ± 1.16[b]	14.5 ± 2.19[b]	9.5 ± 1.19[b]	6.0 ± .25[b]	13.4 ± 1.04[b]	18.0 ± 1.89[b]	11.7 ± 1.16[b]	7.5 ± 0.23[b]

observed in the plants treated with PBZ at the later developmental stages, such as anthesis and post-anthesis.

The final stage of leaf development is inevitable senescence with a decline in physiological activity. Physiological activities of flag leaves in four wheat genotypes were characterized by RWC, P_N, g_s, and maximal photochemical efficiency of PSII. The stability of photosynthetic components could be attributed to a maintenance of leaf RWC under stress as a result of osmotic adjustment. In the present investigation, drought stress caused a marked reduction in P_N and g_s in all wheat genotypes studied. However, the drought-tolerant HD2987 and C306 genotypes were superior to the drought-sensitive HD2888 and HD2733 genotypes with respect to gas-exchange attributes (Fig. 2). Drought-induced reduction in photosynthesis has been reported earlier also in a number of crops including wheat (El-Hafid *et al.*1998) and maize (Ali and Ashraf 2011). The improvement in photosynthetic characteristics under water stress due to PBZ application was also reported by Berova and Zlatev (2003). Percentage increase and decrease of traits (RWC, Chl, P_N, g_s, quantum yield of PSII and LA) due to water stress and PBZ application is given as supplementary figure (Fig. 1S, *supplement available online*).

Proline content and *pyrroline-5-carboxylate synthase* (*P5CS*) gene expression: The proline content was highly sensitive to water-stress conditions in all cultivars of wheat studied (Table 3). Under the control conditions, the mean proline content was the highest at the post-anthesis stage [2.62 µmol g^{-1}(FM)], while the lowest proline content was observed at the pre-anthesis [2.01 µmol g^{-1}(FM)] stage. Similarly, the control (irrigated) plants treated with PBZ showed the highest mean proline content at post-anthesis [3.20 µmol g^{-1}(FM)]. The PBZ treatment enhanced the proline content in all four wheat cultivar and at all three developmental stages under the control condition. However, under the water-stress condition, the mean proline content increased 10-fold as compared with the control plants at the pre-anthesis stage. Under water-stress condition, the plants treated with PBZ showed drop of 40% in the mean proline content in comparison to the water-deficient plants without the PBZ treatment. Due to the PBZ application the highest (75.1%) reduction in the proline content was observed in HD2733, followed by HD2987 (59.8%) at the pre-anthesis stage. The similar trend was observed for the PBZ treatment under water-stress conditions at anthesis as well as post-anthesis in all four
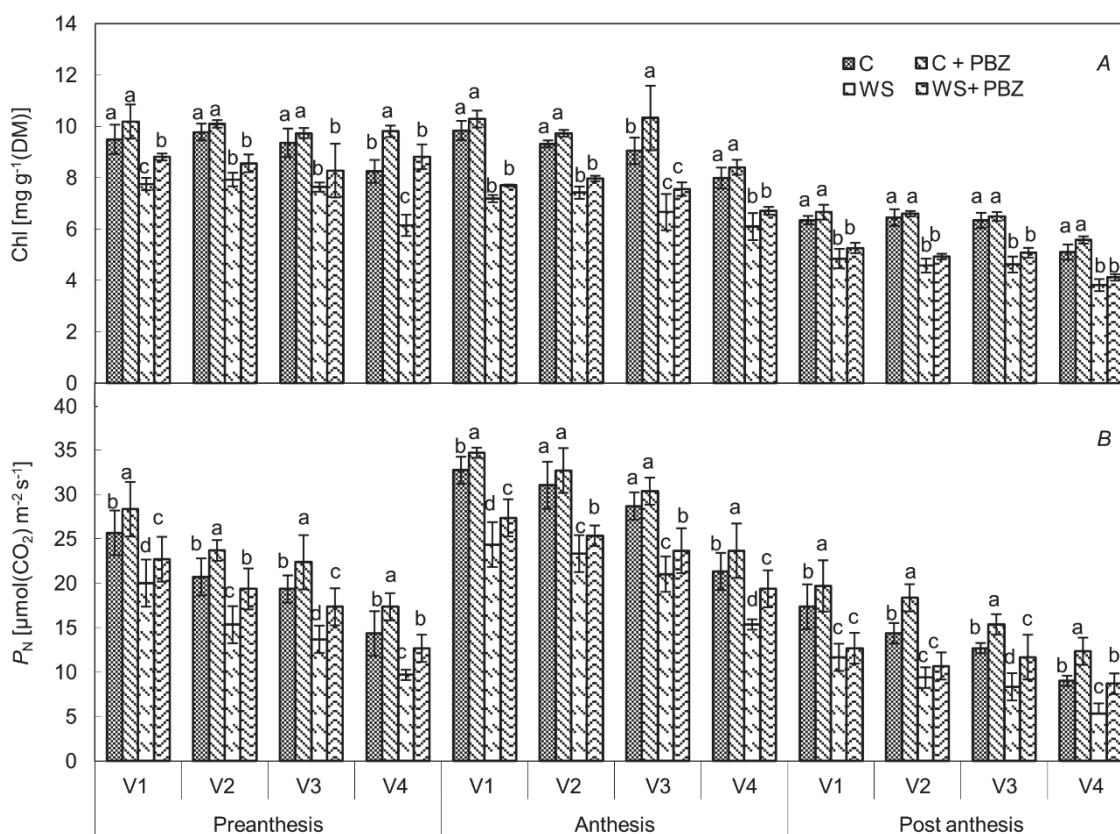

Fig. 1. Effect of paclobutrazol on (*A*) total chlorophyll (Chl) content and (*B*) photosynthetic rate (P_N) at three different growth stages of four wheat genotypes (V1 – HD2987, V2 – C306, V3 – HD2888, and V4 – HD2733) under water-deficit stress. C – control; WS – water stress; PBZ– paclobutrazol. *Different letters* indicate significant differences of Chl content and P_N between treatments in the same year at *p* <0.05.
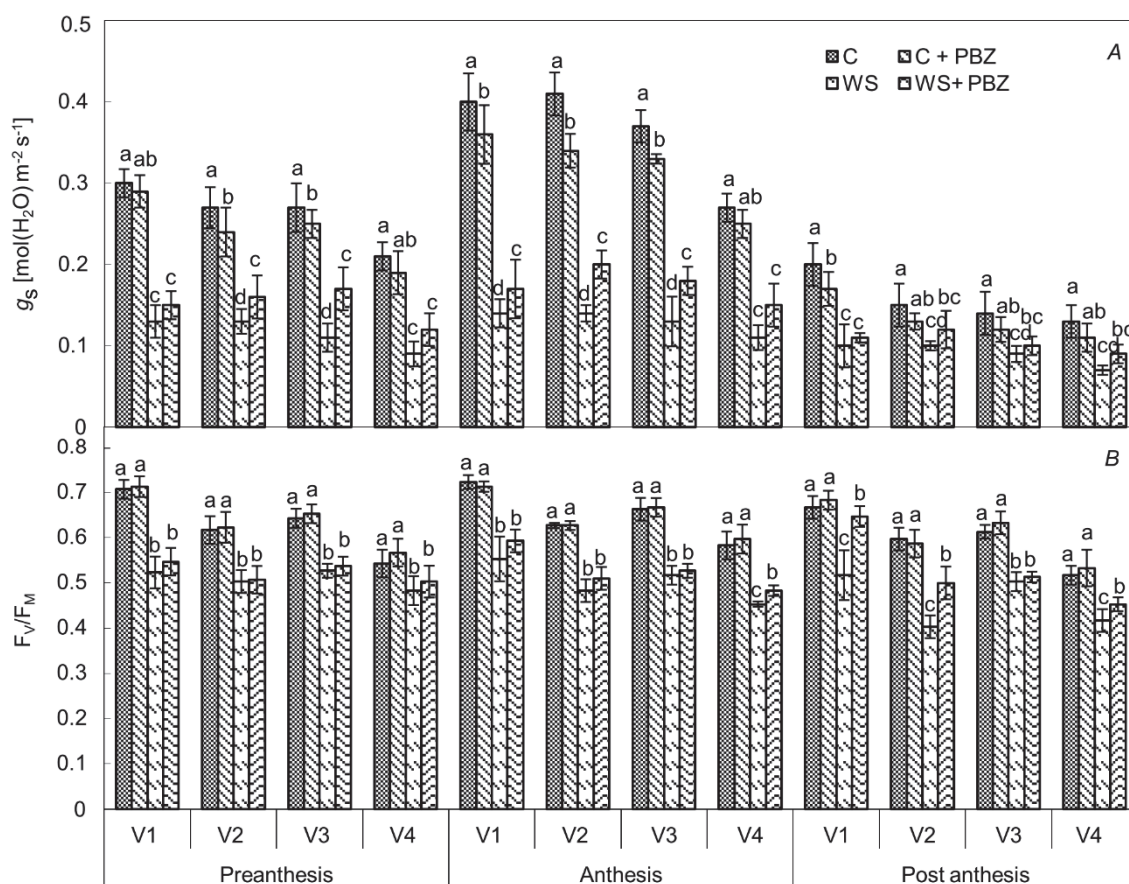
Fig. 2. Effect of paclobutrazol on (*A*) stomatal conductance (g_s) and (*B*) maximal quantum yield of PSII photochemistry (F_V/F_M) at three different growth stages of four wheat genotypes (V1 – HD2987, V2 – C306, V3 – HD2888, and V4 – HD2733) under water-deficit stress. C – control; WS – water stress; PBZ – paclobutrazol. *Different letters* indicate significant differences of g_s and F_V/F_M between treatments in the same year at *p* <0.05.

genotypes of wheat studied. Similar results were also found by various researchers in wheat (Keyvan 2010), and in sugarcane (Batool *et al*. 2012). In response to osmotic stress, many plant species accumulate proline due to a simultaneous abscisic acid-mediated activation of its biosynthesis and inactivation of its degradation pathways during stress (Hare *et al*. 1997).

Expression analysis (level of expression) of the *P5CS* gene was studied at two developmental stages (anthesis and post-anthesis) of wheat genotypes with actin used as internal control (Fig. 2S, *supplement available online*). The highest expression of the *P5CS* gene was found at the post-anthesis stage in all genotypes of wheat studied under water-stress conditions. The control plants treated with PBZ showed the least significant difference in expression of the *P5CS* gene both at anthesis and post- anthesis. The water-stressed plants with the PBZ treatment showed downregulation of *P5CS* at both the stages of wheat development in all the genotypes studied. However, the sensitive genotypes (HD2733 and HD2888) treated with PBZ showed a higher difference in the expression at post-anthesis compared to the plants without the PBZ treatment. Further study also showed that expression of the water-

stress marker gene *P5CS* was upregulated during water stress. However, the application of PBZ downregulated the expression of *P5CS* gene under water stress. Drought stress-induced overexpression of this gene was also reported by Debnath (2008). This finding is also in agreement with the Jungklang *et al*. (2015) who also reported that the accumulation of proline in *Curcuma* leaves could possibly play a protection role apart from osmoregulation during drought stress. However, PBZ might act as a stress-ameliorating agent in this plant, as this species does not need to accumulate proline in leaves. However, the application of PBZ might cause that the wheat genotypes were able to sense a lower stress level; thus, it caused the downregulation of the proline content during water stress.

In conclusion, the water stress caused a significant inhibition of leaf gas exchange. Moreover, the photo-synthetic rate decreased mainly due to the limited access of carbon dioxide to mesophyll cells. However, RWC, P_N, and the F_V/F_M ratio were improved in the PBZ-treated plants with their lower conductance. The application of PBZ (30 μl l^{-1}) led to downregulation of the proline content under water stress, while there was no significant

change in its content under irrigated condition with or without the PBZ treatment. Moreover, the susceptible genotype (HD2733) was more responsive toward the PBZ treatment as compared to those tolerant (HD2987) ones. The higher photosynthesis of flag leaves in the tolerant genotypes might be related to the greater chloroplast

activity under water deficit. This finding indicate that due to the application of PBZ, the negative effect of water stress on the wheat genotypes was minimized *via* the improvement of water status, photosynthetic characteristics, and sensing of lower stress level by plants.

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