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Improving Barley Yield Grown Under Water Stress Conditions

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Abstract

A field experiment was conducted to investigate the influences of paclobutrazol (PBZ) on leaf water potential (ψ_w), proline content, activities of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT), grain yield and water use efficiency (WUE) of barley 'cv. Giza 124' plants subjected to water stress. Plants were treated with two regimes of irrigation water, i.e., 100% of evapotranspiration (ETc) (control) and 60% of ETc and three levels of PBZ solution (0.0 (control), 20 and 40 mg l^{-1}). Leaf water potential, proline content, activities of SOD and CAT, grain yield and WUE were significantly altered by both water stress and PBZ treatments. Results indicated that PBZ (40 mg l^{-1}) mitigated the water stress and significantly reduced the reduction in leaf ψ_w as compared to non-PBZ-treated water-stressed plants. Water-stressed plants treated with PBZ (40 mg l^{-1}) had significant higher proline content than water-stressed plants without PBZ treatment. Higher antioxidant enzyme activity was also observed in water-stressed plants treated by PBZ than water-stressed plants without PBZ treatments. In comparison to water-stressed plants without PBZ treatment, water-stressed plants treated with PBZ (40 mg l^{-1}) had significant higher SOD and CAT activities. Furthermore, water-stressed plants treated with 40 mg l^{-1} of PBZ had also significant higher grain yield and WUE as compared to water-stressed plants without PBZ treatment. The results suggest that PBZ application under water stress conditions alters the equilibrium between free radical production and enzymatic defense reactions in barley by enhancing the proline content and free radical scavenging capacity.

Keywords: Barley, water stress, paclobutrazol, leaf water potential, proline content, superoxide dismutase, catalase, water use efficiency.

Introduction

Under optimal growth conditions, reactive oxygen species (ROS) including H_2O_2, O_2^- and OH^- are continuously produced at low levels mainly in chloroplast, mitochondria and peroxisomes of plant cells. The balance between production and removal of ROS are controlled by cellular osmo-protectants[1] and antioxidant enzyme systems[2]. Whereas, under severe abiotic stress conditions, the reductive enzymatic pathways in plants may be overwhelmed and result in damage of cell components and finally death of plant[3,4]. Attempts have been made in the past to overcome the adverse effects of drought by using plant growth regulators, which have potential to mitigate the water stress. Paclobutrazol, a derivative of triazole group is now commercially used in many tropical and sub-tropical field and vegetable crops for regulation of growth, flowering and yield[5-7]. PBZ interferes with gibberellins biosynthesis by inhibiting the oxidation of ent-kaurene to ent-kauronoic acid through inactivating cytochrome P 450-dependent oxygenase. With respect to its other functions, PBZ has been used to provide plant protection against water stress[8-10]. PBZ has biochemical effects on plants, such as detoxification of active oxygen[11,12] and reduced the decrease in leaf ψ_w[13] and increased levels of proline[14], antioxidants[15] and chlorophyll contents[16]. Based on these studies, the present investigation was conducted to discover the influence of PBZ on drought tolerance in barley, and to determine the interactive impacts of water stress and PBZ on grain yield and WUE in addition to leaf ψ_w, proline content, antioxidant enzyme activities and their possible role in reducing water stress in barley.

Material and Methods

Plant material and treatments: Barley seeds 'cv. G 124' were planted in 10.5 m^2 plots on the 3rd of December 2009. The seeds were sown at a row spacing of 15 cm at 75 kg $feddan^{-1}$. All the agronomic practices, except the irrigation were applied as commonly used in growing barley crop. All plants were fertilized with the recommended N fertilizer (75 kg N $feddan^{-1}$) as urea (46 %N) and 50 kg $feddan^{-1}$ P_2O_5 as ammonium nonphosphate. Loamy soil having pH (1:2, w/v, soil and water solution) 7.40, EC (1:2, w/v, soil and water solution) 1.23 dSm^{-1}, $CaCO_3$ 6.12% and organic matter 1.26% was used in this experiment. All other recommended cultural practices were followed.

The experiment was laid out in completely randomised split design, having two irrigation water regimes (3000 m^3 $feddan^{-1}$ $growing\ season^{-1}$; 100% of ETc (control) and 1800 m^3 $feddan^{-1}$ $growing\ season^{-1}$; 60% of ETc) and three levels of PBZ 0.0 (control), 20 and 40 mg l^{-1}. Irrigation treatments were isolated with 2 m fallow land to avoid the lateral movement of water

during irrigation. Doses of PBZ and irrigation water regimes were selected on the basis of preliminary studies conducted using a barley pot experiment in 2008 (data not shown).

The data were analysed using analysis of variance (ANOVA), as suggested by[17]. Valid conclusions were drawn only on significant differences between treatment means at the $P \leq 0.05$ of probability. The three levels of PBZ were applied as foliar spray to run off 3 times; 30, 45 and 60 days after planting.

The two irrigation water regimes were applied as a percentage of the crop evapotranspiration (ETc). The daily ETo was computed according to the equation of FAO-PM[18] as follows:

$$ETo = \frac{0.408 \cdot \Delta(R_n - G) + \gamma \cdot \frac{900}{T_{mean} + 273} \cdot u_2 \cdot (e_s - e_a)}{\Delta + \gamma(1 + 0.34 \cdot u_2)}$$

Where: Δ = slope of the saturation vapor pressure curve at air temperature (kPa $^0C^{-1}$), R_n= net radiation at the crop surface ($MJm^{-2}\ d^{-1}$), G = soil heat flux density ($MJm^{-2}\ d^{-1}$), Γ = psychometric constant = $0.665\times10^{-3}\times P$, kPa $^0C^1$ (Allen *et al.,* 1998), U_2 = wind speed at 2 m height ($m\ s^{-1}$), e_s = saturation vapor pressure (kPa), e_a = actual vapor pressure (kPa), $(e_s - e_a)$ = saturation vapor pressure deficit (kPa) T_{mean} = mean daily air temperature at 2m height (^0C).

The average daily ETo at Fayoum region was estimated using the monthly mean weather data of Fayoum meteorological station. The average of daily ETo at Fayoum was 3.88, 3.69, 4.73, 7.14 and 9.86 ETo mm/day at December, January, February, March and April, respectively.

The crop water requirements (ETc) were estimated using the crop coefficient according to the following equation:

$$ETc = ETo \times Kc$$

Where: *ETc* =crop water requirements, mm/day. *Kc* =crop coefficient.

The lengths of the different crop growth stages were 20, 50, 60, and 30 days for initial, crop development, mid-season and late season stages, respectively. The crop coefficients (Kc) of initial, mid and end stages were 0.30, 1.15 and 0.25, respectively according to[18].

The amount of irrigation water applied to each plot during the irrigation regime was determined by using the equation given below:

$$IWA = \frac{A \times ETc \times Ii}{Ea \times 1000} + LR$$

Where: *IWA* = irrigation water applied, (m^3), *A* = plot area, (m^2), *ETc* =crop water requirements, (mm/day), *Ii* = irrigation intervals, (day), *Ea* = application efficiency, (%) and *LR* = leaching requirements (m^3).

The amount of irrigation water applied (IWA) was controlled through plastic pipe (spiles) of 50 mm diameter. One spile per plot was used to convey water for each plot. The amount of water delivered through a plastic pipe was calculated according to the following equation[19].

$$Q = CA\sqrt{2gh} * 10^{-3}$$

Where: *Q* = discharge of irrigation water, ($l.\ sec^{-1}$), *C* = coefficient of discharge, *A* = cross section area of irrigation pipe, (cm^2), *G* = gravity acceleration, ($cm.\ sec^{-2}$), *H* = average effective head of water, (cm).

Leaf water potential (ψ_w): Leaf ψ_w was determined using the tissue weight change method[20]. Half g leaves were placed in sucrose solutions of 0.1-1 molal concentration for an hour. Thereafter, the tissues were removed and blotted to remove excess solution and reweighed. The percentage weight gain or loss was plotted against the solute potential of the sucrose solution (1 molal sucrose had a solute potential of -2.69 MPa at 25ºC). Leaf ψ_w is estimated as equivalent to the osmotic potential of the solution in which there is no gain or loss in weight. Osmotic potential (ψ_s) of each sucrose solution was calculated using the Van`t Hoff equation:

$$\psi_s = - C\gamma RT$$

where: C = The molal concentration, γ = The activity coefficient (a value of 1 for neutral solutes such as sucrose in dilute solution), R = The gas constant (0.00831 kg M Pa $mol^{-1}\ ºK^{-1}$), T = The absolute temperature (ºK = ºC + 273).

The change in weight was calculated as a percentage of the original weight and plotted against ψ_s of sucrose solutions. The leaf ψ_w is estimated as equivalent to the ψ_s of the solution in which there is no change in weight (the intercept on the abscissa).

Proline content: Proline content in 10-week-old barley leaves was measured by rapid colorimetric method as suggested by[21]. Proline was extracted from 0.5 g of dry leaf samples by grinding in 10 ml of 3% sulphosalicylic acid and the mixture was then centrifuged at 10,000 ×g for 10 min. Two ml of the supernatant was added into test tubes to which 2 ml of freshly prepared acid-ninhydrin solution was added. Tubes were incubated in a water bath at 90 ºC for 30 min. The reaction was terminated in ice-bath. The reaction mixture was extracted with 5 ml of toluene and vortexed for 15 s. The tubes were allowed to stand at least for 20 min in darkness at room temperature to allow the separation of toluene and aqueous phase. The toluene phase was then carefully collected into test tubes and toluene fraction was read at 520 nm. The proline concentration in the sample was determined from a standard curve using analytical grade proline and calculated on dry weight basis.

Antioxidant enzyme activity: Leaves of 10-week-old plants were excised rapidly weighed (1.0 g fresh weight) and ground with a pestle in an ice-cold mortar with 10 ml 50 mM phosphate buffer (pH 7.0). The homogenates were centrifuged at 20,000 ×g for 30 min at 4ºC. The supernatant filtered through two layers of cheese-cloth were used for the assays of enzymatic activities.

The SOD activity was determined according to the method of[22]. One enzyme unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition in the rate of nitro-blue-tetrazolium (NBT) at 560 nm. The reaction mixture (3 ml) contained 50 mM phosphate buffer (pH 7.0), 200 mM methionine, 1.125 mM NBT, 1.5 mM EDTA, 75 M riboflavin, and 10–40 µl of enzyme extract. Riboflavin was added as the last component. The tubes were shaken and placed 30 cm below a light bank consisting of two 15-W fluorescent tubes. The reaction was started by switching on the light and allowed to run for 10 min, and switching the light off stopped the reaction. The tubes were then immediately covered with black cloth and the absorbance was spectrophotometrically measured at 560 nm. The non-irradiated reaction mixture had zero absorbance (log A_{560}), which was plotted as a function of the volume of the enzyme extract in the reaction mixture. The volume of the enzyme extract producing 50% inhibition of the reaction was read from the resultant graph.

The CAT activity in leaves of 10-week-old plants was determined by employing the method suggested by[23]. CAT activity was assayed by estimating the residual H_2O_2 by oxidation with $KMnO_4$ titrimetrically. The enzyme extraction was done in a similar way to SOD extraction. The reaction mixture consisted of 3 ml of phosphate buffer (0.1 M, pH 7.0), 30 µl of H_2O_2 (5 mM) and 1 ml of enzyme extract. It was then incubated in a test tube at 20 ºC for 1 min, reaction stopped by adding 10 ml of 0.35 M H_2SO_4 and the residual H_2O_2 estimated by titrating the reaction mixture against 0.01 M $KMnO_4$. The end-point for the titration was a faint purple colour which persisted for at least 15 s. A blank was prepared by adding enzyme extract to an acidified solution of reaction mixture at zero time. The enzyme activity was expressed as moles of H_2O_2 10 min^{-1} g^{-1} of fresh weight of leaves.

Fruit yield and water use efficiency (WUE): Total grain yield was recorded at harvest time after the extraction and air-drying grains. WUE values as kg grains m^{-3} of applied water were calculated for different treatments after harvest according to the following equation[24].

$$WUE = \frac{\text{Grain yield (kg/ha)}}{\text{water applied (m}^3\text{/ha)}}$$

Results and Discussion

Leaf water potential (ψ_w): Considerable variation in leaf ψ_w was observed among the treatments of both water stress and PBZ (table 1). Water-stressed plants without PBZ showed a significant reduction in leaf ψ_w than non-water-stressed plants without PBZ. The application of PBZ significantly increased the leaf ψ_w in water-stressed plants. The maximum leaf ψ_w was observed in PBZ (40 mg l^{-1}) as compared to the control (PBZ 0.0 mg l^{-1}). The data pertaining to interaction effect of water stress and paclobutrazol clearly indicated indefective role of PBZ for improving leaf ψ_w and the higher dose of paclobutrazol (40 mg l^{-1}) was found to be more efficient in mitigating the stress by increasing the leaf ψ_w. Water-stressed plants with PBZ (20 mg l^{-1} and 40 mg l^{-1}) had significant reductions in the decrease in leaf ψ_w than water-stressed plants without PBZ treatments (table 1). It is worth mentioning here that as compare to non-water-stressed and non-PBZ-treated plants (control), the water-stressed plants without PBZ treatment showed a significant reduction in leaf ψ_w whereas application of PBZ (40 mg l^{-1}) in water-stressed plants significantly reduced this reduction in leaf ψ_w. Thus, it appears that PBZ has indefective role for improving leaf ψ_w in barley cv. Giza. In this study, the plants treated with PBZ appear to have been more resistant to water stress than those without PBZ treatment, as shown by the alleviation of the reduction in leaf ψ_w. The reduction in the decrease in leaf ψ_w for the PBZ (40 mg l^{-1}) treated plants was particularly significant. Similar results have been reported for bean[5], pea[6], oak[25] and apple[26]. The PBZ influence was, however, reduced the decline in leaf ψ_w. Moreover, upregulation of stress protective bio-molecules in PBZ-treated plants have also enhanced the capacity to limit the damage caused by species of reactive oxygen.

Proline content: Irrespective of PBZ treatment, proline content in water-stressed plants was significantly higher than non-water-stressed plants. The interaction effect of water stress and paclobutrazol treatments was significant, and maximum proline content was observed in water-stressed plants with PBZ (40 mg l^{-1}) as compared to non-PBZ-treated water-stressed plants (table 1). It was found that under non-water-stressed condition PBZ does not have any significant effect, however under water-stressed condition PBZ (40 mg l^{-1}) treatment resulted in a significant increase in proline. This increased level of proline might have resulted more reduction in the decrease in leaf ψ_w of barley leaves due to its osmo-protectant nature; consequently the significant increase in yield was yielded. Similar observations were also made by[11] in cucumber[27], in *Cajanus cajan* and[28] in *Vetiveria zizanioides*.

Antioxidant enzymes activities (SOD and CAT): SOD activity varied significantly in response to the water stress and paclobutrazol treatments. Water stress-treated plants had significantly higher SOD activity than non-water-stressed plants. Similarly, PBZ-treated plants had significantly higher SOD activity than non-PBZ-treated plants. The interaction effect of water stress and paclobutrazol on SOD activity showed maximum activity in water-stressed plants treated with PBZ (40 mg l^{-1}), which was significant as compared to the water-stressed plants without PBZ treatment. However, the minimum SOD activity was noted in non-water-stressed plants without PBZ treatment (control) (table 2). It was also evident that water-stressed plants having paclobutrazol supplements showed significantly higher SOD activity than water-stressed plants without PBZ treatment. SOD catalyses the dismutation of superoxide anion radicals (O_2^-) with great efficiency resulting in the production of H_2O_2 and O_2[29,30] which improves the scavenging systems of cell and reduces the accumulation of free radicals.

The CAT activity in leaves of barley cv. 'Giza 124' followed almost similar trend as in SOD. CAT activity was significantly higher in water-stressed plants than non-water-stressed plants, regardless of PBZ treatment. CAT activity was also significantly influenced by PBZ and it increased with the increasing PBZ dose. Irrespective of water stress treatment, the two doses of PBZ (20 and 40 mg l^{-1}) significantly increased the CAT activity than non-PBZ-treated plants. Interaction effect of water stress and paclobutrazol was found significant. The higher CAT activity was noticed in water-stressed plants with PBZ (40 mg l^{-1}) than water-stressed plants without PBZ (table 2). This clearly suggests the role of paclobutrazol in increasing the level of CAT activity in barley cv. 'Giza 124' under both water-stressed and non-water-stressed conditions. CAT enzyme is an important antioxidant system that catabolises hydrogen peroxide, a precursor of reactive oxidants[31] and reacts with H_2O_2 directly to form water and oxygen[29,30].

Water-stressed plants having paclobutrazol supplements showed higher SOD and CAT activities than water-stressed plants without PBZ treatment. Results clearly suggest the positive role of PBZ in upregulating the SOD and CAT activities in barley cv. 'Giza 124' under water stress. Similar effects of PBZ in increasing the antioxidant enzymes activities (SOD and CAT) have also been observed by other studies[32,15,33-36] had also reported that PBZ-treated plants have very efficient antioxidative defense mechanism for detoxifying and scavenging of toxic oxygen species through an adoptive mechanism involving upregulation of antioxidative enzymes such as SOD and CAT. Therefore, the results in barley are consistent with the findings by[16] who suggested that triazole compounds induce stress tolerance in plants due to increased antioxidant activity.

Fruit yield and water use efficiency (WUE): Regardless PBZ treatment, grain yield feddan^{-1} and WUE of water-stressed plants were significantly lesser than non-water-stressed plants. Irrespective of water stress treatment, grain yield feddan^{-1} and WUE of PBZ-treated plants were significantly higher than non-PBZ-treated plants. The level 40 mg l^{-1} of PBZ was more efficient in this regard. The interaction effect of water stress and paclobutrazol treatments was significant, and maximum WUE was observed in water-stressed plants with PBZ (40 mg l^{-1}) as compared to non-PBZ-treated water-stressed plants (table 3). It was found that under non-water-stressed condition, PBZ does not have any significant effect, however under water-stressed condition PBZ (40 mg l^{-1}) treatment resulted in a non-significant decrease in grain yield feddan^{-1}.[37] demonstrated that sclerophyllous plant, *Phillyrea latifolia*, was able to increase WUE by reducing transpiration losses during midday drought treatment. However, many studies observed that WUE improved with limited water availability. For example[38,39], reported that the WUE of soybean was improved by mild soil water deficits. On the contrary the WUE of vegetable amaranth, a C_4 crop, was unaffected by drought[40]. In this study, this parameter was significantly increased by PBZ (40 mg l^{-1}) treatment under water-stressed condition. This circumstance highlights that barley plants treated with 40 mg l^{-1} of PBZ reduced their irrigation water needs and had significantly higher WUE under droughts and water shortages, thus increasing their suitability for cultivating in semi-arid and arid climatic conditions[41].

Results showed that paclobutrazol (40 mg l^{-1}) minimizes the negative effects of water stress (60% ETc) with evidence of enhancing leaf water potential by upregulating the endogenous production of proline and antioxidant enzymes like SOD and CAT leading to maximization of fruit yield accompanied with higher WUE.

Table-1
Influence of paclobutrazol (PBZ) and water stress on leaf water potential (ψ_w) (MPa) and proline content (µg g^{-1} DW) of 10-week-old barley plants

Water regime ha^{-1}	PBZ conc. (mg l^{-1})	Ψ_w	Proline
100% ETc (3000 m^3 season^{-1})	0	- 0.62	33.77
	20	- 0.60	33.98
	40	- 0.59	34.54
60% ETc (1800 m^3 season^{-1})	0	- 1.69	61.27
	20	- 1.39	62.62
	40	- 1.06	94.01
LSD0.05		- 0.14	6.19
Means of ETc	100% ETc	- 0.60	34.10
	60% ETc	- 1.38	72.63
LSD0.05		- 0.09	4.94
Means of PBZ concentrations (mg l^{-1})	0	- 1.16	47.52
	20	- 1.00	48.30
	40	- 0.83	64.28
LSD0.05		- 0.11	5.52

Table-2
Influence of paclobutrazol (PBZ) and water stress on superoxide dismutase; SOD activity (Units $10min^{-1}$ mg^{-1} protein) and catalase; CAT activity (µmol H_2O_2 $10min^{-1}$ g^{-1} FW) of 10-week-old barley plants

Water regime ha^{-1}	PBZ conc. (mg l^{-1})	SOD	CAT
100% ETc ($3000 m^3$ $season^{-1}$)	0	15.74	30.13
	20	17.20	37.70
	40	18.30	43.65
60% ETc ($1800 m^3$ $season^{-1}$)	0	17.32	33.98
	20	19.40	40.67
	40	20.74	48.61
LSD0.05		1.59	3.84
Means of ETc	100% ETc	17.08	37.16
	60% ETc	19.15	41.09
LSD0.05		1.22	3.10
Means of PBZ concentrations (mg l^{-1})	0	16.53	32.06
	20	18.30	39.19
	40	19.52	46.13
LSD0.05		1.34	3.35

Table-3
Influence of paclobutrazol (PBZ) and water stress on grain yield (ton $feddan^{-1}$) and water use efficiency; WUE (kg m^{-3}) of 10-week-old barley plants

Water regime ha^{-1}	PBZ conc. (mg l^{-1})	Grain yield	WUE
100% ETc ($3000 m^3$ $season^{-1}$)	0	1.731	0.577
	20	1.733	0.578
	40	1.736	0.579
60% ETc ($1800 m^3$ $season^{-1}$)	0	0.442	0.246
	20	0.766	0.426
	40	1.618	0.899
LSD0.05		0.148	0.09
Means of ETc	100% ETc	1.733	0.578
	60% ETc	0.942	0.524
LSD0.05		0.125	0.05
Means of PBZ concentrations (mg l^{-1})	0	1.087	0.412
	20	1.250	0.502
	40	1.677	0.739
LSD0.05		0.133	0.06

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