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RESPONSE OF ANTIOXIDATIVE ENZYMES IN BREAD WHEAT UNDER WATER STRESS AND EXOGENOUS GLYCINE BETAINE

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ABSTRACT

The experiment has been conducted in a greenhouse at the University of Mosul - Republic of Iraq, to study the effect of Glycine betaine(GB) on stressed and water-balanced wheat, and the study included three factors: the first factor is two cultivars of wheat, the second factor is water stress using (PEG-6000) (0, -0.6,-1.2 MPa), the third factor is GB (0,100 mmol l^{-1}), and the following traits were studied: CAT, SOD, APOX and RWC in the flag leaf, plant yield, Dry plant weight and harvest index%. The Exogenous of GB improved the resistance of two cultivars to water stress and led to A significant increase in RWC, CAT, SOD, APOX, grain yield and Dry plant weight in the absence and presence of water stress, and GB encourages plants to trend in seed formation and convert the substances they produce into grains. Water stress caused an increase in CAT, SOD, APOX, decreasing RWC.

INTRODUCTION

Wheat *Triticum aestivum* L. is one of the most important cereal crops around the world, on which the bulk of the world's population depends as their basic vital food, and many countries suffer from low yields per unit area (FAO, 2023). Iraq is increasingly suffering from the effects of climate change, especially the cultivation of wheat, which depends on rainwater (Nosir, 2023), Farmers' ability to adapt to climate change is difficult because many methods are financially expensive (Nofiu and Baharudin, 2024). Although some farmers carry out supplementary irrigation operations to resist water stress, heat stress still negatively affects wheat productivity in Iraq (Ahmed *et al*, 2023), The drought also affected the fertilization operations of the wheat crop, as the drought reduced the efficiency of fertilizers (Hassan, 2022), However, in agricultural systems, wheat plants face different stress conditions, such as: salinity, drought, heavy metals, high and low temperatures, radiation, and nutritional disorders that limit crop productivity, these stressors produce undesirable effects on plant growth and development. Exposure to various Abiotic stresses during the plant's life cycle led to an excessive accumulation of reactive oxygen species, thus occurring oxidation of lipids and membrane proteins. Furthermore, these stresses reduce cell physiological activity including efficient photosynthesis and protein synthesis which can be due to osmotic stress and nutritional imbalance

(Hasanuzzaman *et al*, 2019). Substances that give plants resistance to water stress include many enzymatic antioxidants., such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), guayacol peroxidase (POX), peroxidoxinate (Prxs), and ascorbate cycle enzymes (AsAGSH), such as ascorbate peroxidase (APX), monohydrocorbate reductase (MDHAR), and reductase Dihydroscorbate (DHAR) and glutathione reductase (GR) (Szőllősi, 2014; Bhattacharjee, 2019). Glycine betaine GB in plants is a natural product that is biosynthesized in plant tissues in response to abiotic stress (lack of water, soil flooding, high and low temperature, UV, metal poisoning, salinity), glycine betaine is synthesized in chloroplasts and plays a major role in protecting the photosynthetic system from the stresses of drought (Ashraf and Foolad, 2007). Plants begin with some defense mechanisms in order to cope with stress; one of them is associated with changes in metabolites. glycine betaine and proline, two cytosol-regulating compounds that are synthesized by many plants in response to stress, including salinity stress, help maintain the osmotic state of the cell to mitigate the effect of abiotic stress (Chinnusamy *et al*., 2005; Vinocur and Altman, 2005). Numerous researches have shown that glycine Betaine stimulates the production of antioxidant enzymes (Wang *et al*, 2010; Gupta, 2015; Ma *et al*, 2006), wheat varieties differ in their glycine production rate (Khan *et al*, 2012), and glycine betaine concentration increases when plants are exposed to environmental stress (Giri, 2011). This study aims to explore the effect of drought stress and glycine betaine on the production of oxidative acids in two cultivars of wheat and the effect of external treatment with glycine betaine on oxidative antigens.

MATERIALS AND METHODS

The experiment was conducted in A greenhouse at the College of Agriculture and Forestry/ University of Mosul - Republic of Iraq, to study the effect of glycine betaine on stressed and water-balanced wheat plants, the present study included three factors: first factor two cultivars of wheat (Baghdad1, Buhuth 22), second factor water stress using polyethylene glycol (PEG-6000) (0, -0.6, -1.2-MPa), and the third factor was glycine betaine (0, 100 mmol l^{-1}). Seeds were soaked, and plants were sprayed until fully wet at the SZ31 stage. Planting occurred on 12/3/2022, and tap water was used for irrigation. The experiment followed a Randomized complete block design (RCBD) with three blocks. The experimental unit consisted of 7 pots with dimensions (220×300×180 mm), each containing 7 kg of clay loam soil texture (EC 2.5, pH 7.4) and sown with five seeds. Duncan's multiple range test was used to compare the means.

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The following traits were studied: catalase (CAT), superoxide dismutase (SOD), Ascorbate peroxidase (APOX) and leaf relative water content (RWC) were estimated in the flag leaf in stage ZS61, Plant yield (g) This is done by calculating the yield of all plants in the pots and extracting the average yield of the individual plant, Dry plant weight (g) by drying the plants at a temperature of 105°C until the weight stabilizes, and harvest index % for the pot were measured According to Kemanian *et al* (2007).

CAT activity was measured spectrally according to Aebi (1983). Sheets (10 mm diameter) were truncated in 1 mL 50 mM insulating phosphate (pH 7.0) with polyvinyl polyprolidone in microcentrifugal tubes. The extracts were centrifuged at 16,000 gn for 15 minutes. The reaction volume 3 ml (1.5 ml of crude extract and 1.5 ml 30 mM H_2O_2). A reduction in absorption was observed at 240 nm in kinetic mode during the degradation of H_2O_2, and the shape of the peak was the shoulder of the peak. APOX activity was measured from the same crude extract as CAT, excluding no dilution. The oxidation of ascorbate was determined by the decrease in absorption at 290 nm as described by Mittler and Zilinskas (1991), and the shape of the peak was the shoulder of the peak.

Enzyme extraction and examination for SOD extraction Leaf samples (0.5 g) were homogenized in a cold 0.1 m phosphate solution (pH 7.5) containing 0.5 mM EDTA with a pre-cooled pestle and slurry. Each sample is homogeneous was transferred to centrifugal tubes and centrifuged at 4 °C for 15 minutes at 15,000×. The floating material was used to examine enzyme activity (Esfandiari *et al*., 2015). SOD activity was determined according to (Gupta *et al*. 1993) by measuring the reduction inhibition of NBT (nitroplotetrazolium) at 560 nm. One enzyme unit was defined as the amount of enzyme that can cause a 50% inhibition of the photochemical reaction, and the shape of the peak was the broadening of the peak. The RWC was measured according to the following equation RWC (%) = [(W-DW) / (TW-DW)] × 100, (Arndt *et al*., 2015).

RESULTS AND DISCUSSION

Relative leaf water content (RWC)

Baghdad1 excelled significantly in RWC, achieving 78.8% Table (1). Water stress caused a continuous and significant decrease as the stress level increased. The addition of GB improved RWC, with a performance of 76.7%, and there was a significant interaction between the cultivars and water stress. Notably, the Baghdad1 cultivar showed greater resistance to water stress. There was a significant difference in the interaction between the two cultivars with GB, as Baghdad1 had the most pronounced response, achieving 80.7% with the addition of GB. GB mitigated the effects of water stress on RWC, as evidenced by the significant interaction between water stress and GB. The highest significant RWC rate, 87.7%, was achieved by adding GB to Baghdad1 under no water stress, observed in the triple interaction.

Catalase (CAT)

We found that the CAT levels in Baghdad1 were significantly higher than in Buhuth 22, with Baghdad1 reaching 4.8 μmol. This explains the resistance of the Baghdad1 cultivar to water stress. This is also evident from the significant interaction between water stress and the cultivars Table (1). We also found that GB increased the CAT content in the cultivars. There was a significant interaction between the cultivars and GB, as GB boosted the CAT levels under increasing water stress. This may be due to GB's role in protecting catalase enzymes.

Table (1): Impact of Glycine betaine, water stress, and cultivars on RWS, CAT, SOD, APOX, grain yield, dry plant weight (g), and harvest index (HI%).

Treatment	RWC%	CAT activity (μmol H2O2 reduced gFW-1)	SOD activity (mg protein–1)	APOX (Mmol. g^{-1} fresh weight)	Grain yield (g. Pot^{-1})	Dry plant weight (g. Pot^{-1})	HI %
Cultivar (CV)							
- Baghdad1	78.8 A	4.8 A	73.1 A	80.4 A	37.9 A	100.5 A	37.6 B
- Buhuth 22	71.2 B	4.5 B	69.3 B	76.3 B	36.1 B	87.2 B	41.6 A
Water Stress (WS)							
- 0 MPa	84.2 A	4.4 C	65.5 C	72.1 C	42.6 A	103.4 A	41.2 A
- 0.6 MPa	77.9 B	4.7 B	71.5 B	78.6 B	35.9 B	92.5 B	39.1 B
- 1.2 MPa	62.9 C	4.9 A	76.7 A	84.4 A	32.5 C	85.6 C	38.6 C
Glycine betaine (GB)							
- 0 mmol l^{-1}	73.4 B	4.4 B	70.2 B	77.2 B	35.7 B	93.1 B	38.3 B
- 100 mmol l^{-1}	76.7 A	4.9 A	72.3 A	79.5 A	38.3 A	94.6 A	40.9 A
Interaction CV*WS							
- Baghdad1*0 MPa	85.6 A	4.5 D	67.3 D	74 E	43.7 A	105.1 A	41.6 B
- Baghdad1*0.6 MPa	79.6 C	4.9 A	73.3 B	80.6 C	36.8 C	100.8 B	36.5 D
- Baghdad1*1.2 MPa	71.2 E	4.9 A	78.8 A	86.7 A	33.3 E	95.6 C	34.8 E
-Buhuth 22*0 MPa	82.8 B	4.3 C	63.8 E	70.2 F	41.5 B	101.8 B	40.8 C
-Buhuth 22*0.6 MPa	76.2 D	4.6 B	69.7 C	76.7 D	35 D	84.1 D	41.7 B
-Buhuth 22*1.2 MPa	54.7 F	4.8 A	74.6 B	82.1 B	31.8 F	75.7 E	42.4 A
Interaction CV*GB							
- Baghdad1*0 mmol l^{-1}	76.9 B	4.5 B	72 B	79.2 B	36.6 C	97.8 B	37.4 C
- Baghdad1*100 mmol l^{-1}	80.7 A	5 A	74.2 A	81.6 A	39.2 A	103.1 A	37.9 C
- Buhuth 22*0 mmol l^{-1}	69.8 D	4.2 C	68.3 D	75.2 D	34.8 D	88.4 C	39.3 B
- Buhuth 22*100 mmol l^{-1}	72.6 C	4.9 A	70.3 C	77.4 C	37.4 B	86 E	43.9 A
Interaction GB*WS							
- 0 mmol l^{-1}*0 MPa	82.4 B	4.1 D	64.6 F	71.1 F	41.1 B	99.7 B	41.2 A
- 0 mmol l^{-1}*0.6MPa	76.2 D	4.4 D	70.4 D	77.5 D	34.5 D	92 C	37.6 B
- 0 mmol l^{-1}* 1.2 MPa	61.6 F	4.6 C	75.6 B	83.2 B	31.6 F	87.7 D	36.2 B
- 100 mmol l^{-1}*0 MPa	86.1 A	4.7 C	66.5 E	73.1 E	44.2 A	107.2 A	41.2 A
- 100 mmol l^{-1}*0.6 MPa	79.6 C	5 A	72.6 C	79.8 C	37.3 C	93 C	40.6 AB
- 100 mmol l^{-1}*1.2 MPa	64.3 E	5.1 A	77.8 A	85.6 A	33.5 E	83.6 E	41.0 A
Interaction CV*WS*GB							
- Baghdad1*0*0	83.5 B	4.2 C	66.3 H	72.9 H	42.1 B	101.7 C	41.4 C
- Baghdad1*0.6*0	77.6 D	4.6 B	72.2 E	79.4 E	35.4 G	97.5 D	36.3 G
- Baghdad1*1.2*0	69.5 G	4.7 B	77.6 B	85.4 B	32.4 H	94.2 DE	34.4 I
- Baghdad1*0*100	87.7 A	4.8 B	68.2 G	75 G	45.3 A	108.4 A	41.8 C
- Baghdad1*0.6*100	81.5 C	5.1 A	74.4 D	81.8 D	38.2 E	104.1 B	36.7 F
- Baghdad1*1.2*100	72.9 F	5.1 A	79.9 A	87.9 A	34.1 GH	96.9 D	35.2 H
- Buhuth 22*0*0	81.2 C	3.9 D	62.9 J	69.2 J	40 C	97.6 D	41.0 C
- Buhuth 22*0.6*0	74.7 E	4.2 C	68.6 G	75.5 G	33.6 H	86.4 E	38.9 D
- Buhuth 22*1.2*0	53.6 I	4.5 CB	73.5 D	80.9 D	30.8 I	81.1 F	38 E
- Buhuth 22*0*100	84.4 B	4.6 B	64.7 I	71.2 I	43 B	105.9 B	40.6 C
- Buhuth 22*0.6*100	77.7 D	4.9 B	70.7 F	77.8 F	36.3 F	81.8 F	44.4 B
- Buhuth 22*1.2*100	55.7 H	5.1 A	75.6 C	83.2 C	32.8 H	70.2 G	46.7 A

Superoxide dismutase (SOD)

The SOD content in the Baghdad1 cultivar was significantly higher, reaching 73.1 mg. SOD levels increased continuously and significantly with rising water stress. GB also contributed to the increase in SOD concentration as water stress intensified. This is evident from the interaction between water stress and GB, as well as the triple interaction Table (1). Additionally, GB raised the SOD content in both cultivars, with both responding positively to the addition of GB.

Ascorbate peroxidase (APOX)

Baghdad1 cultivar showed a significant result, achieving 80.4 mmol g^{-1} fresh weight. This significance continued in the interaction between cultivars and water stress, as well as in the triple interaction Table (1). The interaction of Baghdad1 with the addition of GB reached the highest significant value of 80.7 mmol.g^{-1} fresh weight. Additionally, Baghdad1's interaction without water stress resulted in 85.6 mmol.g^{-1} fresh weight. Water stress caused a continuous and significant increase in APOX content as the intensity of water stress increased.

Grain yield (g Pot^{-1})

Baghdad1 produced a significant Grain yield of 37.9 g Pot^{-1}. This is attributed to the superiority of Baghdad1 in RWC, CAT, SOD, and APOX. Water stress caused a continuous and significant decrease in yield; however, GB can mitigate this decrease. This effect is evident in the triple interaction and the interaction between water stress and GB Table (1). The Baghdad1 cultivar is more resistant to water stress, as observed in the triple interaction and the interaction between cultivars and water stress. This resistance is due to the significant performance of Baghdad1 in RWC, CAT, SOD, and APOX.

Dry plant weight (g Pot^{-1})

We found that Baghdad1 had a significant dry plant weight, achieving 100.5 g. The dry plant weight was affected by water stress, which caused a significant decrease. However, the addition of GB increased the dry plant weight in both cultivars and reduced the negative effects of water stress. The effect of GB can be inferred from the significant interaction between GB and water stress, as well as the triple interaction.

Harvesting index % (HI%)

The cultivar Buhuth 22 excelled significantly, achieving 41.6%. This is due to the fact that Buhuth 22 produced a higher grain ratio to dry plant weight. However, it yielded less grain, indicating inefficiency in transporting and producing substances that feed the sink. This inefficiency is also observed with increased water stress Table (1). GB improved the harvest index (HI%), as its addition resulted in a significant increase in HI%, reaching 40.9%. GB mitigates the negative impact of water stress, which is evident from the interaction between water stress and GB, as well as the triple interaction.

CONCLUSIONS

The application of exogenous glycine betaine enhanced the cultivars' resistance to water stress, resulting in increased levels of RWC, CAT, SOD, and APOX. Glycine betaine contributed to higher grain yield and dry plant weight both under water stress and without water stress conditions. Additionally, it promotes grain formation and facilitates the conversion of produced substances into grains.

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CONFLICT OF INTEREST

Both authors acknowledge and undertake that this work does not conflict with the interests of others.

استجابة انزيمات مستضدات الاكسدة في حنطة الخبز تحت الاجهاد المائي والمعاملة بالجلايسين بيتان

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الخلاصة

أجريت التجربة في بيت زجاجي تابع لكلية الزراعة والغابات/جامعة الموصل – جمهورية العراق، لدراسة تأثير الجلايسين بيتان على نباتات القمح المجهدة والمتزنة مائيا، وتضمنت الدراسة ثلاث عوامل؛ العامل الأول صنفين من القمح والعامل الثاني الاجهاد المائي باستخدام (PEG–6000) (0، 0.6–، 1.2– MPa)، والعامل الثالث الجلايسين بيتان (0، 100 ملي مول لتر$^{-1}$)، وتم دراسة الصفات الاتية: CAT و SOD و APOX و RWC في ورقة العلم، وتم قياس حاصل النبات، اوزن النبات الجاف ودليل الحصاد%. إضافة الجلايسين بيتان حسنت من مقاومة الأصناف للإجهاد المائي وأدت الى زيادة معنوية في RWC, CAT, SOD, APOX، وحققت زيادة معنوية في حاصل الحبوب والحاصل الحيوي في غياب ووجود الاجهاد المائي، كما ان الجلايسين بيتان يشجع النباتات على الاتجاه في تكوين البذور وتحويل المواد التي ينتجها الى الحبوب. الاجهاد المائي تسبب في زيادة CAT، SOD، APOX، في حين RWC انخفض، وتفوق معنويا الصنف بغداد1 على بحوث 22 في جميع الصفات المدروسة.

الكلمات المفتاحية: الاجهاد المائي، أصناف الحنطة، بولي اثلين جلايكول–6000، الجلايسين بيتان، SOD.

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