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Glycine Betaine Improves Growth, Antioxidants and Yield of Barley (*Hordeum vulgare* L) Grown in Sandy Soil

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ABSTRACT

Pretreatment strategies are used to enhance crop growth and yield in sandy soils. This study aims to investigate the effects of glycine betaine (GB) as a grain priming agent on growth parameters, yield components, and metabolic changes in barley (*Hordeum vulgare* L.) at various concentrations: 0.0, 5, 10, and 20 mM. The application of glycine betaine resulted in a significant enhancement in the growth and yield of barley. Conspicuously, a concentration of 10 mM GB significantly increased the activity of antioxidant enzymes. Furthermore, chlorophyll content, total soluble sugars, phenolic compounds, total flavonoids, and amino acids were markedly elevated at 20 mM GB, while levels of proline and hydrogen peroxide (H_2O_2) were reduced. Additionally, the protein profile exhibited GB-responsive patterns that varied with concentration. The enhanced antioxidant capacity, modified protein profiles, and biochemical constituents of barley underscore the effectiveness of osmo-priming with glycine betaine in promoting growth, metabolic activities, and yield parameters in barley plants cultivated in sandy soil.

Keywords: Antioxidant Enzymes; Barley; Glycine betaine; Growth Enhancement; *Hordeum vulgare;* Osmo-Priming; Proline.

INTRODUCTION

Barley *(Hordeum vulgare* L.) crop belongs to cereal plants and is considered a strategic crop worldwide. It is used for malting, bread making, animal feeding, and adding to wheat flour in certain regions. Additionally, barley exhibits greater tolerance to drought and poor soil conditions compared to other cereals, making it essential for cultivation in arid regions. The barley crop is grown in coastal locations and the reclaimed soils under various irrigation techniques (Hussein, 2022). Identifying viable strategies to enhance plant development and yield under climate changes and sandy soil environments is vital.

Many factors can influence the growth of barely plants, such as soil, environment, and cultivation techniques. Cultivation in sandy lands that is confronted with a number of difficulties, including a lack of soil fertility, a restriction on water availability, and an increase in salinity (Hussein *et al.,* 2019). In many crops, the grain priming technique improves grain vigor and germination synchronization (Du *et al.,* 2019). Soaking periods depend on plant species in which grain hydrations must reach a certain level before activating of germination process (Lutts *et al.,* 2016). The previous works have highlighted the grain priming techniques to enhance biosynthesis of growth signals, early DNA replications, increased ATP biosynthesis, osmotic adjustments, and membrane reorganization through restoring their primary structures and minimizing leakage of metabolites (Hussein *et al.,* 2022; Johnson and Puthur, 2021) . Plants may also control their growth and development through the primary (peptides, carbohydrates, amino acids, proteins) and secondary (total phenols, total flavonoids) metabolites that they synthesized (Niu *et al.,* 2023). Glycine betaine (GB) is osmoregulatory substance widely presented in plants (Hasanuzzaman *et al.,* 2019). GB is a natural and organic metabolite that exhibits a crucial role in osmoregulatory processes due to its low viscosity and high -water solubility. In addition to its osmo-protective properties, it stabilizes the structure of enzymes and proteins, strengthens antioxidant systems, decreases the membrane permeability and hydrogen peroxide (H_2O_2) mediated signaling (Islam *et al.,* 2021). In many plant species, GB typically accumulates in the chloroplasts, mitochondria and cytosol, and these accumulations increase in response to stressors (Ahmed *et al.,* 2021). It is a safe organic substance that maintains the efficiency and the structure of photosystem(PS II) in chloroplast, which increases photosynthetic efficiency (Chen and Murata, 2011). Similarly, as glycine betaine builds up in crops, the nitrogen it contains enhances root formation and seed germination, ultimately leading to plant growth (He *et al.,* 2011). Previous studies showed that using GB as foliar (Ahmed *et al.,* 2019; Athar *et al.,* 2015) or root (Islam *et al.,* 2021) applications can enhance a plant's physiology and development under unstress and stress conditions. For instance, under normal conditions, foliar spray of GB enhanced water status, proline, CO_2-fixation, stomatal conductance and water efficiency, and to promoting crop development (Athar *et al.,* 2015; Ahmed *et al.,* 2019a; Islam *et al.,* 2021). Therefore, in order to address the problems provided by climate change and resource restrictions, this work intends to investigate novel agronomic approaches and treatments that enhance the growth, production, and stress resilience of barley *(Hordeum vulgare* L.) in sandy soils. In particular, it focuses on osmo-priming with glycine betaine (GB) to improve the production and growth of

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barley planted in sandy soil. It also aims to understand the mechanisms by which GB helps to overcome the difficulties related to sandy soils.

MATERIALS AND METHODS

Growth conditions

During the winter of 2017-2018, pots experiment was conducted at greenhouse of the faculty of Science (Girls-Branch), Al-Azhar University, Egypt. The objective of the work was to evaluate the impact of glycine betaine pretreatment on the growth, biochemical composition, and yield of barley (*Hordeum vulgare* L.) plants. The Agriculture Research Center (ARC), Giza, Cairo, Egypt, provided the barley grains (Cultivar: Giza 123). The grains were soaked for 12 h in different concentrations of GB (0, 5, 10 and 20 mM). Six replicates of each treatment were planted in pots (40x40) filled with sandy soil; five plants per pot. Sandy soil with a field capacity (FC) of 11.6%, EC 0.34 dSm^{-1}, pH 8.6, HCO_3^-1.00%, Cl^-1.7%, K^+ 0.26%, Na^+ 1.2%, and Ca^{++} 1.27% Mg^{++} 0.60 $meqL^{-1}$ were the characteristics of the soil. Fertilizers containing calcium superphosphate and potassium sulfate were applied prior to seeding. After thirty- and sixty-days following planting, nitrogen was applied as ammonium nitrate. Each pot was irrigated every week (1.5 L; 80% FC).

Morphological parameters

Representative samples were taken 65 days after planting from each treatment to measure the growth traits: plant height, root length, number of leaves per plant, leaf area, and the fresh and dry weights of shoot and root per plant. At harvest time, the yield parameters were determined, including the number of tillers $plant^{-1}$, number of spikes $plant^{-1}$, yield of spikes $plant^{-1}$, yield $plant^{-1}$, weight of 1,000 grains, and straw weight per plant.

Photosynthetic pigments

According to (Metzner *et al.*, 1965), photosynthetic pigments were determined in fresh barley leaves in 85% acetone. Fresh weight (100 mg) of barley leaves was extracted in the acetone solvent. The extracts were centrifuged at 1000 xg, and the supernatants were up to (10 ml acetone, 85%). The absorbance was at 663 nm, 644 nm, and 452 nm on spectrophotometer against a blank (acetone). The pigment (chlorophyll a, chlorophyll b, and carotenoids) was expressed as µg/ml according to the next equations: Chlorophyll-a=10.3 E663 - 0.918E644= μgml^{-1}, Chlorophyll-b=19.7E644 - 3.870E663= μgml^{-1}, Carotenoids = 4.2 E452 - (0.0264 chlorophyll a + 0.426 chlorophyll b) = μgml^{-1}. The photosynthesis pigments were represented as mgg^{-1} FW of barley plant.

Total soluble sugars

Using the anthrone approach, the total soluble sugars in the ethanolic extract of dried tissues of the barley plant were calculated (Cerning, 1975). TSS were measured by 0.1 ml of ethanol extract was used in the reaction with 3.0 ml of freshly made anthrone (0.15g anthrone and 100 ml H_2SO_4, 72%) in a water bath for ten min, after cooling the samples, absorbance was read at 625 nm.

Total free amino acids

Free amino acids (FAA) in dried tissue of pretreated barley plants were measured by the ninhydrin reagent according to (Rosen, 1957) The extracts were prepared using 80.0% ethanol. Following centrifugation, 1 ml of extract was mixed to 0.5 ml buffer [27 g, sodium acetate, distilled H_2O (20 ml), glacial acetic acid (5 ml), 490 ppm NaCN (1.5 ml) and the solution up to 75 ml with distilled water (pH = 5.4), followed by (0.5 ml of ninhydrin reagent)]. Then, the solution was put in a boiling water bath for 15 min. 5 ml of 50% isopropanol was added after cooling. The absorbance was measured at 570 nm. Free amino acids were expressed as mgg^{-1} DW, using L-glutamic acid standard curve.

Proline content

Proline was detected in fresh leaves (0.5 g) using the method of Bates et al. (1973). The 0.5 g of fresh leaves was extracted in 10 ml of 3% aqueous sulfosalicylic acid to determine the proline concentration in the studied plant samples. A total of 2 ml of the extract, 2 ml of acid-ninhydrin reagent, and 2 ml of glacial acetic acid (CH_3COOH) were combined and boiled at 100 ºC for 1 hour. After cooling, the mixture was combined with 4 ml of toluene to extract the proline content. The absorbance was measured at 520 nm using a spectrophotometer, with toluene serving as a blank.

Total flavonoids and phenolics compounds

Total flavonoids in dry tissues of barley crop were measured according to Adom and Liu, (2002). Two milliliters of diluted extract were added to 0.2 ml of 5% $NaNO_2$, allowed to react for five min, and then mixed with 0.2 ml of 10 % $AlCl_3$. The catechin served as a reference for measuring the absorbance at 510 nm. The technique outlined by Savitree *et al.* (2004) and Pourmorad *et al.* (2006) was used to estimate the total phenolic contents in dry leaves. One milliliter of extract and 10.0 drops of conc hydrochloric acid, in a boiling water bath for 10 min. The mixture was cooled and combined with 1.5 ml of Na_2CO_3 (14%) and one ml of Folin-Ciocalteau reagent. The mixture was thoroughly shaken with rising to 5ml of distilled water; it was placed in a water bath for 5.0 min. The absorbance was recorded at 650 nm and the data was expressed as mgg^{-1} DW.

Antioxidant enzymes activity

In accordance with the test of various enzyme activities, the crude enzyme was extracted. A barley leaf (2 g) was isolated and stored at 4˚C for a night in 10 ml of 100 mM phosphate buffer, pH 6.8. For ten min, the extract was centrifuged at 5000 xg. Enzyme activities were measured using the supernatant as a crude extract (Mukherjee and Choudhuri, 1983).

Peroxidase (POD)

POD activity was assayed according to (Bergmayer, 1974). Phosphate buffer (5.8 ml, 50 mM, pH 7.0), pyrogallol (2.0 ml, 20 mM), and H_2O_2 (2.0 ml, 20 mM) reacted with a 0.2 ml crude extract. Using a

spectrophotometer, absorbance against an enzyme-free reagent at 470 nm was measured in 60 sec. Enzyme activity unit is equal to the quantity of crude enzyme needed to convert one µ mole of hydrogen peroxide in a minute at room temperature (Kong *et al.*, 1999).

Ascorbate peroxidase (APX)

APX assay was according to (Koricheva *et al.*, 1997). Using a UV-VIS spectrophotometer, the rate of absorbance drop as ascorbate oxidized was measured at 290 nm. The quantity of enzyme needed to catalyze the change of one µ mole of H_2O_2 min^{-1} was used to determine one unit of enzyme activity (ε= 2.8 mM^{-1} cm^{-1}) at 25°C.

Catalase (CAT)

CAT activity was assayed according to (Chen *et al.*, 2000). Enzyme extract (40 µl) and phosphate buffer (pH 7.0, 9.96 ml) containing H_2O_2 (0.16ml of 30% H_2O_2 in 100 ml of 50 mM phosphate buffer) were added to the reaction mixture, which had a final volume of 10 ml. Using a spectrophotometer, the rate at which H_2O_2 absorbance changed in a minute relative to a buffer blank at 250 nm was used to calculate the CAT activity. Rather than employing enzyme extract, buffer was used to create the blank sample. The quantity of enzyme that decreased 50 % of H_2O_2 min^{-1} at normal temperature is equivalent to one unit of enzyme activity.

Hydrogen peroxide (H_2O_2)

The H_2O_2 content was determined at 390 nm using the method described by Velikova et al. (2000), and the absorbance was recorded. H_2O_2 concentrations were expressed as nmol g^{-1} FW, using an extinction coefficient (ε) of 0.28 μm^{-1} cm^{-1}.

Protein profile

The rapid freeze-dried (200 mg) leaf tissues were extracted with one ml buffer, and stored in the freezer for 1 hr, and shaken for 15 sec, then centrifuged at (5000 xg) for 15 min at 4˚C. After that, SDS-PAGE, or sodium dodecyl sulphate-polyacrylamide gel electrophoresis, was carried out in accordance with (Laemmli, 1970). Using standard protein markers (11-180 kDa), the molecular weight of the separated proteins was calculated. Following documentation, the protein bands were observed using Coomassie Brilliant Blue G-250 staining (Sigma, USA).

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) using Minitab® 18.1 Statistical Data Document. The results were statistically analyzed according to Snedecor and Cochran., (1989). To compare means, the Tukey (HSD) test was calculated at a 5% probability level. The data were presented as mean ± standard error (n = 3).

RESULTS

Effect of GB on growth parameters

The data presented in the table (1) show the effects of varying concentrations of Glycine Betaine on several growth parameters of plants. The parameters assessed include plant height, root length, the number of leaves per plant, leaf area per plant, and fresh and dry weights of both shoots and roots.

The plant height remained relatively stable across the different concentrations of Glycine Betaine, with no significant difference ($p > 0.05$) observed among the treatments. For root length, there was no significant difference in root length between the control (0 mM) and lower concentrations (5 and 10 mM). However, a marked increase is observed at the highest concentration (20 mM), where root length significantly ($p \leq 0.05$) exceeded the other treatments.

The number of leaves per plant and leaf area both exhibited significant positive responses at higher concentrations (20 mM), where the highest values were recorded. At 10 mM and 20 mM, the number of leaves increased by 22.5 % and 36.5 %, respectively over the control. Moreover, GB increased significantly flag leaf area in the GB pretreated plants. The highest increment was represented by 29.1% compared to the other plants. This suggests that GB may promote leaf development and overall plant strength. In addition, shoot fresh weight (FW) recorded an increase with higher concentrations of GB and recorded the highest at 20 mM. Similarly, shoot dry weight (DW) showed an upward trend and was significantly higher at 20 mM compared to the control. The maximum increase (55.5% and 60%) was observed in 10 mM GB-pretreated plants for fresh and dry weights of shoots related to control plants.

For both fresh and dry root weight, a similar trend is observed, with both root fresh weight (FW) and dry weight (DW) being significantly ($p \leq 0.05$) enhanced at higher concentrations of GB compared to lower concentrations and the control. Fresh weight increased by 28.57% and 35.71%, while root dry weight per plant increased by 63.3% and 73.3% in comparison to untreated plants.

Effect of GB on barely yield

Table (2) presents the effects of different concentrations of GB on various growth parameters of barley plant, including the number of tillers, spike number, weight of spikes, weight of grains, weight of 1,000 grains, and weight of straw per plant. The number of tillers increases significantly with GB concentrations and recording the highest at 20 mM (8.8 ± 1.3), where it is statistically higher than all other concentrations. However, spike number shows a mixed response. It is highest at 10 mM (4.50 ± 0.6) but drops to 3.50 ± 0.5 at 20 mM, indicating a possible adverse effect or a non-linear response at higher concentrations. For the spike weight $plant^{-1}$, the weight recorded the highest at 10 mM (5.3 ± 0.20), suggesting that this concentration optimally supports spike development, after which weights decline or do not increase significantly at $p \leq 0.05$. The weight of 1,000 grains shows variability but improves with increasing GB concentrations up to 10 mM (75.29 ± 2.92) before decreasing slightly at 20 mM (69.75 ± 3.07). This trend reflects what was seen in spike and grain weights. Similarly, straw weight recorded the highest weight

Table (1): Assessment of growth parameters in *Hordeum vulgare* L. plants treated with osmo-priming glycine betaine (GB) and cultivated in sandy soil. Data are represented in means ± standard error.

GB Conc. (mM)	Measured growth parameters							
	Plant height (cm)	Root length (cm)	Leaves no plant^{-1}	Leaf area plant^{-1} (cm^2)	Shoot FW plant^{-1} (g)	Shoot DW plant^{-1} (g)	Root FW plant^{-1} (g)	Root DW plant^{-1} (g)
0	66.0±1.2^a	16.0±1.4^b	20.0±1.5^c	22.0±0.5^c	23.8±1.7^c	2.50±0.2^b	1.40±0.1^b	0.30±0.02^c
5	66.4±3.2^a	17.5±2.1^b	22.8±1.2^b	25.2±0.9^b	24.0±3.5^c	2.62±0.2^b	1.50±0.2^b	0.42±0.02^b
10	69.0±1.4^a	19.5±2.3^b	24.5±1.7^b	25.9±0.2^b	27.9±2.0^b	2.92±0.5^b	1.80±0.1^a	0.49±0.02^a
20	71.0±1.2^a	30.0±2.4^a	27.3±1.0^a	28.4±1.0^a	37.0±3.2^a	4.00±0.4^a	1.90±0.3^a	0.52±0.02^a

Means superscripts with different letters, per column, are significantly different at $p \leq 0.05$ based on Tukey (HSD) test.

Table (2): Effect of osmo- priming with glycine betaine (GB) on yield attributes of barley (*Hordeum vulgare* L.) plants cultivated in sandy soil. Data are means ±standard error.

GB conc. (mM)	Measured yield parameters					
	Tillers plant^{-1}	Spike no plant^{-1}	Spikes weight plant^{-1} (g)	Grains weight plant^{-1} (g)	1000 grains weight (g)	Straw weight plant^{-1} (g)
0	3.30±0.6^c	2.01±0.1^c	2.2±0.10^c	2.00±0.30^c	38.21+3.32^c	4.79±0.57^c
5	5.30±0.9^b	3.30±0.4^b	3.50±0.40^b	3.20±0.20^b	50.43+4.09^b	7.34±0.42^b
10	6.00±1.1^b	4.50±0.6^a	5.3±0.20^a	4.60±0.60^a	75.29+2.92^a	8.43±0.54^{ab}
20	8.8±1.3^a	3.50±0.5^b	4.9±0.60^a	4.11±0.40^a	69.75+3.07^a	9.35±1.24^a

Means superscripts with different letters, per column, are significantly different at $p \leq 0.05$ based on Tukey (HSD) test.

at 20 mM GB (9.35 ± 1.24). In conclusion, the number of tillers, spikes, and straw weight per plant exhibited progressive and significant increases in glycine betaine (GB)-pretreated plants compared to the control. The 20 mM GB pretreatment increased the number of tillers and straw weight per plant by 166.7% and 95.2%, respectively, compared to the control. Concerning the number of spikes per plant, weight of spikes, weight of grains per plant, and weight of 1000 grains, the results showed marked increases ($p \leq 0.05$) with increasing GB concentration. The 10 mM pretreatment showed the highest increases in these parameters, reaching 125%, 141%, 130%, and 97%, respectively, compared to the control values.

Photosynthetic pigments

The results depicted in Fig. (1) demonstrated how GB-pretreatment affected the photosynthetic pigments in barley (*Hordeum vulgare* L.) plants. GB pretreatments at 5, 10 and 20 mM caused progressive increase in chl-a, chl-b and carotenoids. Pretreated plants with 20 mM GB achieved the highest increment reaching to 32.24 %, 36.73 %, and 16.89 % compared to the control. Pretreated plants with 5 and 10mM GB showed no marked changes in carotenoids in comparison with the control value.

Total soluble sugars

According to the findings displayed in Figure 2, pretreatments with glycine betaine led to significantly higher levels of total soluble sugars in the barley leaves ($p \leq 0.05$) in comparison to the untreated plants. The highest increase percentage observed in barley plant leaves, originating from grains soaked in 20 mM GB, was 54.6%.

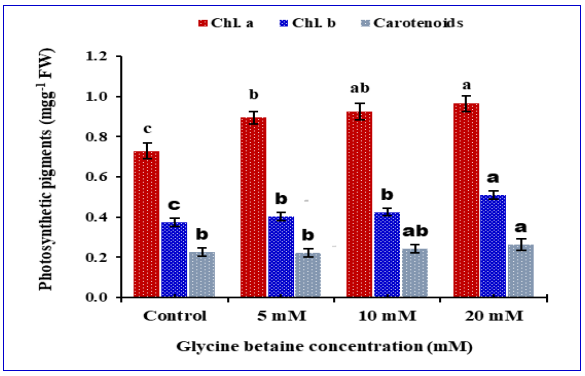

Figure (1): Effect of osmo-priming with glycine betaine on the photosynthetic pigments (mg g^{-1} FW) in barley (*Hordeum vulgare* L.) plants cultivated in sandy soil. Bars for each chlorophyll type with different superscript letters are significantly different at $p \leq 0.05$ based on the Tukey test. Vertical bars represent ± standard deviation (SD).

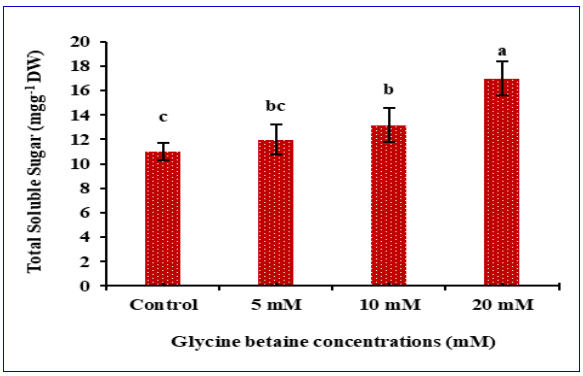

Figure (2): Effect of osmo-priming with glycine betaine on total soluble content of sugars (mg g^{-1} DW). Bars for each chlorophyll type with different superscript letters are significantly different at $p \leq 0.05$ based on the Tukey test. Vertical bars represent ± standard deviation (SD).

Total free amino acids and proline content

The results represented in Figure (3A) indicate that all GB treatments increased the free amino acids (FAA) content compared to the control value. Meanwhile, the plants pretreated with GB exhibited a significant decrease in proline levels (Fig. 3B). The maximum decrease was observed with the 20 mM GB treatment, which reached 40% compared to the control.

Total flavonoids and phenols

Data in Figure (4A) demonstrated a significant increase in total phenols with GB treatments in comparison to the control. The highest increase of phenol content was 62.24 % by 20 mM GB pretreated plants in relation to the control value. In parallel, the obtained value for total flavonoids (Figure 4B) showed that there was a progressive increase in the total flavonoids in barley plants with increasing the concentration of GB. The highest percentages were obtained at 20 mM GB which was reached to 165.7 % in relation to the control value.

Antioxidant enzyme

The activities of catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) were significantly affected by the pretreatment of barley (*Hordeum vulgare* L.) plants with glycine betaine (GB) as represented in Figure (5 A-C). The enzyme activities of CAT, APX, and POD were elevated at various concentrations of GB, except for the 20 mM concentration. Remarkably, the highest increase in enzyme activity was observed at 10 mM GB compared to the control group.

Hydrogen peroxide

Application of GB pretreatments with 5 mM showed non-significant effect on hydrogen peroxide. On the other hand, the endogenous H_2O_2 increased by 41.79% in 10 mM GB pretreated plants (Fig. 5D), while decreasing by 28.21% in 20 mM GB pretreated plants in comparison to the control value.

Protein profile

There were variations in the protein bands due to glycine betaine pretreatment (Fig. 6 and Table 3). Glycine betaine causes alterations in protein profiles through enhancement of the synthesis of different protein bands. Pretreatment with 20 mM induced the unique 20 kDa and 19 kDa polypeptides. The polymorphic polypeptides 65 kDa and 155 kDa were induced in 10 mM and 20 mM pretreated plants. All concentrations of glycine betaine induced a 25 kDa polypeptide. The presence of these protein bands related to glycine betaine treatment of barley grown in

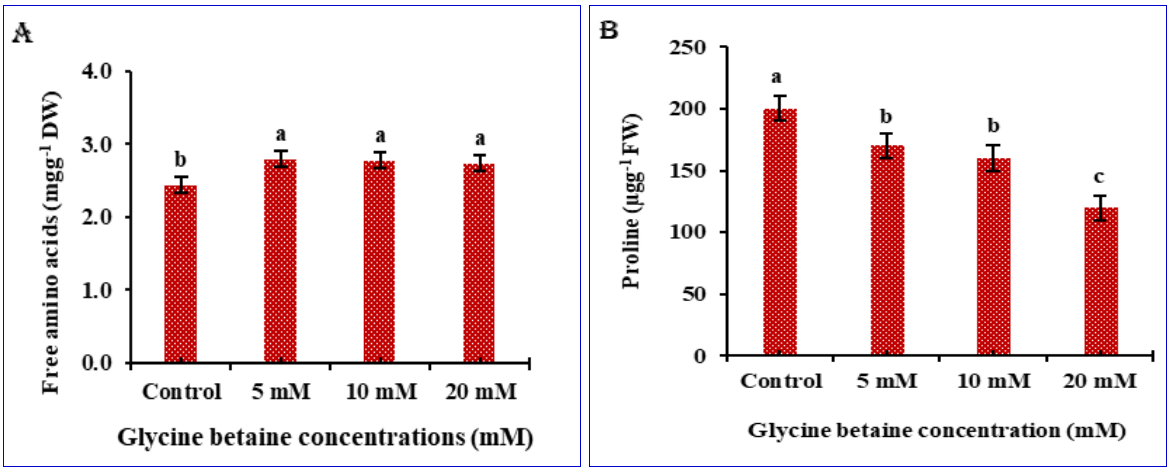

Figure (3): Effect of osmo-priming with glycine betaine on Free amino acids and proline content of barley (*Hordeum vulgare* L.) plants cultivated in sandy soil. A, Free amino acids content (mg g^{-1} DW); B, Proline content (µg g^{-1} FW). Bars with different superscript letters are significantly different at $p \leq 0.05$ based on the Tukey test. Vertical bars represent ± standard deviation (SD).

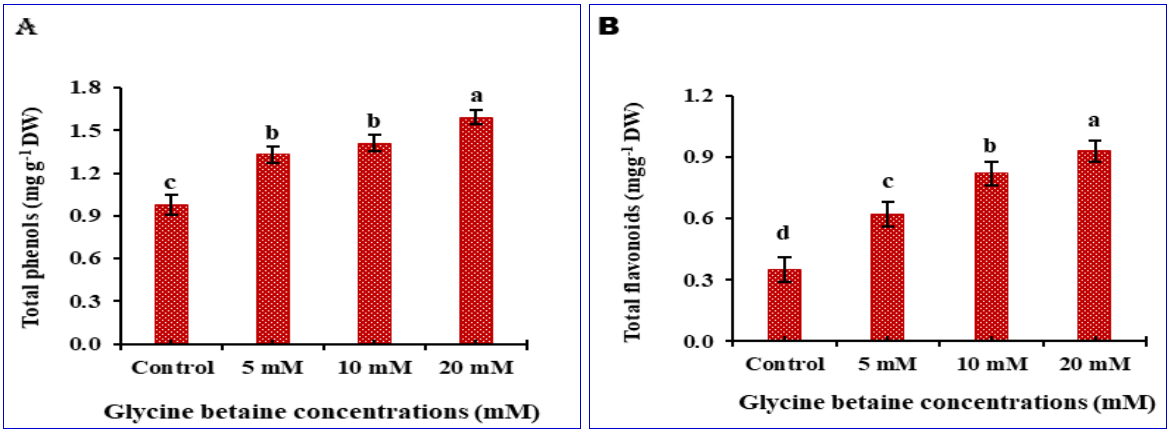

Figure (4): Effect of osmo-priming with glycine betaine on total phenols and total flavonoid of barley (*Hordeum vulgare* L.) plants cultivated in sandy soil. A, Total phenols (mg g-1 DW); B, Total flavinoides (mg g-1 DW). Bars with different superscript letters are significantly different at $p \leq 0.05$ based on the Tukey test. Vertical bars represent ± standard deviation (SD).

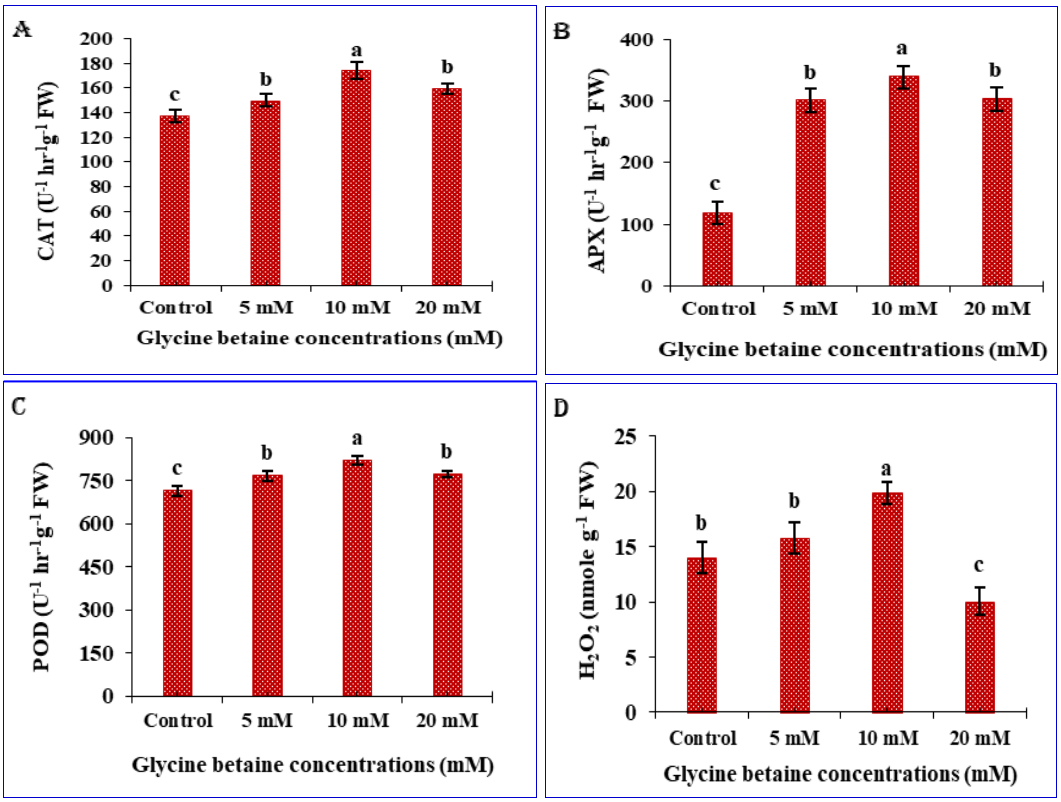

Figure (5): Effect of osmo-priming with glycine betaine on antioxidant activities barley (*Hordeum vulgare* L.) plants cultivated in sandy soil. A, Catalase; B, Ascorbate peroxidase; C, peroxidase activities and D, hydrogen peroxide. Bars with different superscript letters are significantly different at $p \leq 0.05$ based on the Tukey test. Vertical bars represent ± standard deviation (SD).

in sandy soil suggests that the plants may have adaptation responses to the treatment. Additionally, the unique and induced polypeptides observed at different glycine betaine concentrations indicate the same response to GB.

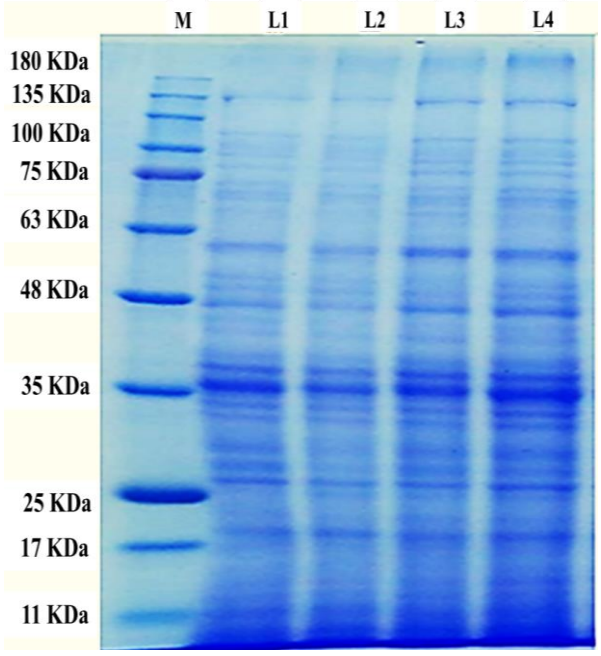

Figure (6): Effect of osmo-priming with glycine betaine (GB) on protein profile in barley (*Hordeum vulgare* L.) plants cultivated in sandy soil. GB concentration were: L1, 0.0 mM; L2, 5 mM; L3,10 mM and L4, 20 mM. M, lader.

Table (3): Effect of osmo-priming with glycine betaine (GB) on proteins profiles in the barley (*Hordeum vulgare* L.) plants cultivated in sandy soil.

No.	MW	GB concentration (mM)			
		0.0 (Control)	5.0	10.0	20.0
1	170	+	+	+	+
2	155	-	-	+	+
3	110	+	+	+	+
4	100	+	+	+	+
5	89	+	+	+	+
6	78	+	+	+	+
7	72	+	+	+	+
8	70	+	+	+	+
9	68	+	+	+	+
10	67	+	+	+	+
11	65	-	-	+	+
12	59	+	+	+	+
13	53	+	+	+	+
14	50	+	+	+	+
15	47	+	+	+	+
16	44	+	+	+	+
17	42	+	+	+	+
18	39	+	+	+	+
19	37	+	+	+	+
20	35	+	+	+	+
21	34	+	+	+	+
22	33	+	+	+	+
23	30	+	+	+	+
24	28	+	+	+	+
25	27	+	+	+	+
26	25	-	+	+	+
27	23	+	+	+	+
28	21	+	+	+	+
29	20	-	-	-	+
30	19	-	-	-	+
31	18	+	+	+	+
32	17	+	+	+	+
33	11	+	+	+	+

+, band exists; -, no band detected.

DISCUSSION

Barley (*Hordeum vulgare* L.) produces edible grains grown in a large scale of environments. Interestingly, with the strategic advantages, crop productivity decreases yearly. It is vital to use recent improvement strategies for increasing productivity. Grain priming with glycine betaine had potential stimulatory effects on improving the development and yield in barley planted in sandy soil. Such potential stimulating effects of glycine betaine on plant development may result from modifications to the antioxidants in crop plants (Chen and Murata, 2011; Sharma *et al.*, 2023).The present results showed improvement in the performance of growth, biochemical, yield components of barley (*Hordeum vulgare* L.) plants cultivated in sandy soil. The obtained data indicated that the GB-pretreated plants exhibited beneficial effects on growth parameters. These GB-priming enhanced growth parameters hence higher value for fresh weights of shoots and roots. Eventually, the higher dry weight would have been the consequence of higher fresh weights. These results agree with the earlier findings of on wheat (Ahmed *et al.*, 2019) and mustard plants (Islam *et al.*, 2021). Such enhancement in these parameters might be attributed to the role of GB on essential metabolic processes (Farooq *et al.*, 2016). The application of GB enhanced amino acids over the control. The stimulated growth of glycine betaine pretreated barley plants might be due to the activation protein biosynthesis during the germination and the hormonal status of endogenous abscisic acid and gibberellin in plants (Zhang *et al.*, 2022). In this respect, foliar application of barley plants with glycine betaine promoted the antioxidant systems (Sharma *et al.*, 2023).

The grains priming with GB also improved yield components in comparison with the control group. In barley, GB appears to promote the metabolism involved in reproductive partitioning. The increased synthesis of dry matter that results from the improvements in chlorophylls, osmolytes, and antioxidant potential will provide the carbon skeleton needed for the improvement of yield attributes (Raza *et al.*, 2014). Our findings generally agree with those of (Raza *et al.*, 2014) on wheat, (Adak and Tozlu, 2020) on strawberry and (Shafiq *et al.*, 2021) on maize. Grain priming with GB enhances the growth parameters associated with an increased antioxidant systems leading to improved yield components of barley plants. Furthermore, the increase in yield components may have been due to the increase in essential amino acids and endogenous GB (Shafiq *et al.*, 2021).

The results of this investigation showed that barley plants have higher levels of photosynthetic pigments. Chl "a", Chl b, and carotenoids are the major precursors of green energies, any change in their values affects the plant metabolic processes (Hussein *et al.*, 2019). Data in the present investigation showed that GB-pretreated barley plants have higher levels of photosynthetic pigments. GB synthesis occurs in chloroplast, so the results could be due to the action of Glycine betaine in stabilizing the oxygen evolving photosystem (PSII) complex in chloroplast against environmental challenges (Rezaei *et al.*, 2012; Jalal-ud-Din *et al.*, 2015). Additionally, this increase in pigment concentrations could have resulted from the function of GB in protection the photosynthetic machinery and maintaining the stability of plant membranes and Rubisco structures (Rezaei *et al.*, 2012; Jalal-ud-Din *et al.*, 2015). Furthermore, it is well known that GB induces the photosynthetic efficiency.

Soluble sugars consider as osmo-protectant molecules, stabilize cell membranes and adjust turgor pressure (Jalal-ud-Din *et al.*, 2015). In the present study, total soluble sugars increased in the GB-pretreated barley plants. Similarly, earlier studies have reported that foliar application of GB, there are enhances in total soluble sugars in cowpea, lettuce and wheat (Badran *et al.*, 2015; Khalifa *et al.*, 2016; Manaf, 2016). The increase in total soluble sugars may be increase and improvement the plant tolerance under stressors.

Amino acids are the building units needed to synthesize proteins and other metabolites. They have a role in biochemical and physiological processes, including ion transport (Quan *et al.*, 2016), osmotic pressure modulation (Farooq *et al.*, 2010), enzyme activity modification, and stomatal opening regulation. Grain priming with Glycine betaine increased total free amino acids but it reduced the proline levels in barley (*Hordeum vulgare* L.) plants, indicating that this substance has a beneficial effect on upregulating the metabolic processes involved in growth and yield under sandy soil. It is obvious that proline is a sensitive molecule in response to applied GB. Proline may be participated in new protein synthesis.

GB regulates variety of metabolic functions in plants under stress and unstress conditions (Athar *et al.*, 2015). For total flavonoids, glycine betaine induces remarkable accumulation in barley plants. Plants may be able to biosynthesize flavonoids due to their stimulating and antioxidant effects on plant development. Total phenolics are a class of secondary metabolites that may be involved in avoiding lipid peroxidation, scavenging reactive oxygen species (ROS), minimizing DNA damage, and denaturation of proteins (Jalal-ud-Din *et al.*, 2015). Total phenols increased in barley plants planted in sandy soil. The application of GB increased total phenols under stressful conditions in maize (Shafiq *et al.*, 2021). The significant positive correlation between applications of glycine betaine and total phenols refers to the antioxidant mechanisms in the pretreated plants. The role of glycine betaine may be due to activating antioxidant enzymes, which regulate phenolic levels.

POD and CAT enzymes enhanced the transformation of H_2O_2 to water and oxygen (Gratão *et al.*, 2005). Antioxidant enzymes and reactive oxygen species balance indicates oxidative damage or maintaining the antioxidant capacity (Møller *et al.*, 2007). The glycine betaine induced the activities of CAT, APX and POD

enzymes more than controls. Glycine betaine may have a modulatory influence on the structure of enzymes, leading to enzyme activation. The induced CAT, POD, and APX enzymes activities indicate the hydrogen peroxide split. It indicated that the alteration in the antioxidant enzymes activities may be related to their protective action in vital processes (Møller *et al.*, 2007). In the study, glycine betaine significantly reduced H_2O_2 content in comparison with the control plants. The higher H_2O_2 level depending on the glycine betaine concentration might be due to scavenge ROS under stress. The results showed that the declining of these biomolecules might be attributed to an enhancement in the antioxidant capacity to scavenge free radicals in plants planted in sandy soil. ROS are produced by oxidative processes in mitochondria and chloroplasts. While their higher level results in cellular oxidative damage, their lower concentration aids in signaling. The exogenous GB reduced the amounts of H_2O_2 generation. The decrease in ROS production mediated by GB may be explained by its ability to scavenge ROS. Therefore, GB reduces ROS formation by protecting cell membranes with inducing osmolytes and antioxidants (CAT, APX, and POX). These findings are in agreement with the previous results of (Ahmed *et al*., 2021; Ahmed *et al.*, 2019).

Priming of the barley grains in glycine betaine induced the biosynthesis of new proteins in green plants. In response to GB-osmopriming, the biosynthesis of new proteins stimulates physiological mechanisms of barley plants, especially at high concentrations. Alterations in protein expression in amino acids treated plants may play a part in essential physiological functions like enzymes synthesis, osmotic balance, membranes stability, electrons transport, and signals transduction (Hussein *et al.*, 2022; Hussein and Alshammari, 2022, Hussein et al., 2023, 2019b). The synthesis of new protein is one of the protective mechanisms of plant in response to amino acid application. Moreover, the effect of amino acids on proteins synthesis might be due to the build of the disulfide bonds between polypeptides lead to the configuration of the low molecular weight proteins (Hussein and Alshammari, 2022).

CONCLUSIONS

Grain priming with GB stimulated growth parameters, yield components, photosynthetic pigments, total amino acids, proteins, phenols, flavonoids, and regulated hydrogen peroxide, proline, and antioxidant enzymes. Therefore, grains osmo-priming with GB is an effective pretreatment strategy that changes metabolic signals which participates in enhancing growth and increasing yield components of barley plants grown in arid and semiarid soil.

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