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Effect of Exogenous Application of Glycinebetaine on Wheat (*Triticum aestivum* L.) Under Heavy Metal Stres

Khizar Hayat Bhatti, Sehrish Anwar, Khalid Nawaz, Khalid Hussain, Ejaz Hussain Siddiqi, Raja usmansharif, Aqsa talat and Aneelakhalid

Department of Botany, Institute of Chemical and biological sciences, University of Gujrat, Gujrat-50700, Gujrat, Pakistan

Abstract: Various strategies may be used to induce enhanced protection against heavy metal stress. Foliar application of GB is an effective approach for imparting tolerance among plants against heavy metals and other abiotic stresses. It scavenges reactive oxygen species (ROS), promotes osmotic adjustment, stabilizes proteins and protects membrane integrity in plants under adverse conditions. In a pot experiment, GB was exogenously applied at 0, 50 and 100 mMconc to wheat plants. Based on data, it is suggested that GB spray increased morphological parameters such as shoot and root length, biomass, photosynthetic pigments and Na^{+}, K^{+} ion contents. Thus, it is stated that exogenous GB application is an applicable approach to deal effectively with heavy metal toxicity in wheat.

Key words: Exogenous · Glycinebetaine · Wheat · Heavy metal · Tolerance

INTRODUCTION

When plants experience the unfavorable conditions such as salinity, low temperature or drought, plant cells protect themselves by accumulating a variety of small organic metabolites that are called compatible solutes [1]. Heavy metal stress stimulates the accumulation of many osmolytes such as glycinebetaine, which help in producing tolerance [2].

GB is a zwitterionic, quaternary ammonium compound and derivative of glycine that is found in a large number of higher plants, animals and microorganisms [3]. It is dipolar in nature, but electrically neutral molecule and highly soluble in water. Due to these characteristics, GB protects or stabilizes 3D structures of enzymes and membranal proteins by interacting with both hydrophilic and hydrophobic domains [4-6]. GB is an important osmolyte in plants and accumulates in cytosol and chloroplast [7, 8]. GB can play an important role in effective protection against drought, high salt concentration and high temperature [9-11]. GB is produced in many plant families including Gramineae, Compositae, Malvaceae, Chenopodiaceae and Amaranthaceae [12, 13]. Exogenous application of GB to enhance stress tolerance ability of different crop plants has got attention of many researchers since many years [14-16]. Foliar application of GB is the suggested way as a shotgun approach to induce tolerance against stress conditions in crops with poor or no solute accumulating ability. Effectiveness of exogenously applied GB depends on a number of factors including type of species, concentration of GB and plant developmental stage at which applied [17].

GB improved salt and drought tolerance in rice [18], maize [19], wheat [20] and tomato [21, 22]. Stabilization of the quaternary structures of enzymes, complex proteins and protection of membranes are important effects of GB [23, 24]. GB also protects physiological processes such as photosynthesis and protein synthesis under drought stress [25, 26]. Plants treated with GB also maintain higher antioxidative enzyme activities and minimize oxidative stress [27]. It also improves germination and seedling growth of many crop plants under stressful environment [28, 29]. GB enhances water utilization efficiency of winter wheat [30].

GB enhances tolerance to high temperatures [31], freezing temperatures [32], drought stress [33], low temperatures [34] and salinity [35, 36].

Corresponding Author: Khizar Hayat Bhatti, Department of Botany, Institute of Chemical and biological sciences, University of Gujrat, Gujrat-50700, Gujrat, Pakistan.

GB induces tolerance by protecting the Rubisco enzyme [37], maintaining turgor pressure [38] and Photosystem II [39]. Foliar application of GB improves the survival rate and growth of plants under a variety of stresses [40]. GB over expression can enhance the salt tolerance of transgenic plants by regulating ion homeostasis [41]. The objective of the study is to observe that the up to what extent foliar application of GB could ameliorate the adverse effects of heavy metals on wheat.

MATERIAL AND METHODS

Two wheat varieties Sehar-2006 and Chakwal-97 were used in the experiment to study the effects of foliar application of GB. Experiment was performed in Botany lab and nursery of University of Gujrat, Hafiz Hayat Campus, Gujrat. Seeds were sterilized with sodium hypochlorite and washed by distilled water. Tap water was used to wash the sand. Seeds of each variety were sown in sand in plastic pots on 27th February 2012. For each variety, 15 plastic pots were used. Tap water was given regularly to plants for irrigation during course of study. GB was applied at 0, 50 and 100 mM solution concentration levels. After one week of GB spray, all growth and biochemical parameters were noted. The experiment was laid down in a completely randomized design (CRD) with 5 replicates (n=5) for each treatment of both varieties. After 15 days of treatment, following parameters were measured.

Determination of Morphological Parameters: Two plants were taken from each replicate to measure morphological parameters. Root length, shoot length were measured of each replicate with the help of scale. Root and shoot fresh weights were also measured with help of analytical balance.Then, they were oven-dried at 65°C for one week and their dry weights recorded. Numbers of tillers were also noted.

Determination of Chlorophyll Contents: Chlorophyll a, chlorophyll b and carotene contents were determined according to Arnon method (1949).0.5 g fresh weight of leaf was ground with 2ml alcohol, then 5 ml more alcohol was added and after keeping overnight in test tubes, OD (optical density) was measured with the help of spectrophotometer.

Chlorophyll a, chlorophyll b and carotenes were measured by following formulas:

- Chlorophyll a: $12.7 \times OD(663) - 2.69 \times OD(645)$ mM
- Chlorophyll b: $22.9 \times OD(645) - 4.68 \times OD(663)$ mM
- Carotenes: $OD(480) + 0.114 \times OD(663) - (0.638) \times OD(645)$ mM

Determination of GB Contents: Dry leaf material (1.0 g) was ground in 10 ml of distilled water and filtered. 1 ml of the extract was mixed with 1 ml of 2M HCl after filtration, then 0.5 ml of this mixture was taken in a glass tube and 0.2 ml of potassium tri-iodide solution was added to it. The contents were shaken and cooled in an ice bath for 90 min with occasional shaking. Then 2.0 ml of ice cooled distilled water and 20 ml of 1-2 dichloromethane (cooled at -10° C) were added to the mixture. The two layers formed in the mixture were mixed by passing a continuous stream of air for 1-2 min while tubes were still in ice bath (4°C). The upper aqueous layer was discarded and optical density of the organic layer was measured at 365 nm. The concentrations of the betaine were calculated against the standard curve.

Determination of Na^+ and K^+ ion Contents: Samples were taken after digestion and got the reading from flame photometer. Standard solutions of different concentration of testing nutrients (Na+, K+) were made. Standards were made of 50,100,150,200,250 ppm. Reading of standards from flame photometer was taken.

Statistical Analysis: Statistical analyses were carried out by using analysis of variance (ANOVA) and Microsoft Excel 2003.

RESULTS

Shoot Length (cm): GB increased the shoot length of both wheat varieties in all the treatments (50 and 100 mM) as compared to control. However, 100 mM GB has significantly (p=0.001) increased the shoot length in both the varieties (Fig.1). Chakwal-97 showed significantly (p=0.001) higher values regarding shoot length than of Sehar-2006 after GB spray application.

Root Length (cm): Results showed that root length of both two varieties increased significantly (p=0.001) with GB foliar application. And there is least significant (p=0.05) variation observed in both varieties regarding root length after GB spray. Chakwal-97 showed slightly more root length than Sehar-2006 (Fig.2).

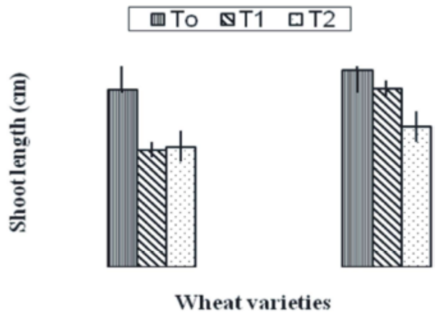

Fig. 1: Shoot length of two wheat varieties after GB exogenous application when plants were 45 days old [T1=50 and T2=100 mM respectively].

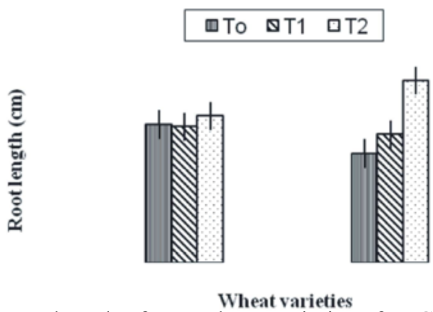

Fig. 2: Root length of two wheat varieties after GB spray when plants were 45 days old [T1=50 and T2=100 mM respectively].

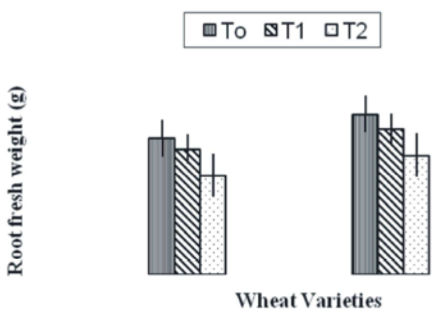

Fig. 3: Root fresh weight of two wheat varieties after GB application when plants were 45 days old [T1=50, T2=100 mM respectively].

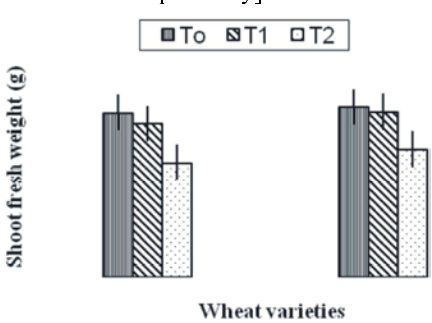

Fig. 4: Shoot fresh weights of two wheat varieties after GB exogenous application when plants were 45 days old [T1=40 and T2=60 ppm respectively].

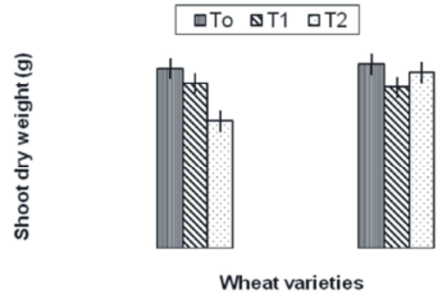

Fig. 5: Shoot dry weight of two wheat varieties after GB application when plants were 45 days old [T1=50 and T2=100 mM respectively].

Fig. 6: Root dry weight of two wheat varieties after GB spray when plants were 45 days old [T1=50, T2=100 mM respectively].

Root and Shoot Fresh Weights: Results showed that root fresh weight was increased after GB application significantly (p=0.01). Chakwal- 97 showed more root fresh weight than Sehar-2006 (Fig. 3). And GB increased the shoot fresh weight at both treatments (50 and 100mM) as compared to control. However, 50mM solution of GB significantly (p=0.001) increased the shoot fresh weight (Fig. 4). There is highly significant variation in both wheat varieties. Chakwal-97 has more shoot fresh weight as compared to Sehar-2006.

Shoot and Root Dry Weights: GB increased shoot dry weight in both varieties however, GB solution of 100 mM significantly (p= 0.001) increased the shoot dry weight. Chakwal-97 had slightly more shoot dry weight as compared to Sehar -2006 (Fig. 5). And GB has increased the root dry weight (g) in both varieties significantly (p=0.001) at both treatments (50 and 100 mM) as compared to control (Fig. 6). There is no significant (ns) variation regarding root dry weight in both varieties.

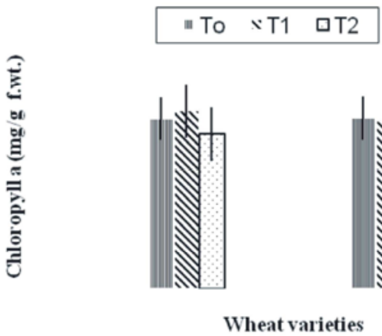

Fig. 7: Chl a contents of two wheat varieties after GB foliar application when plants were 47 days old [T1=50 and T2=100 mM respectively].

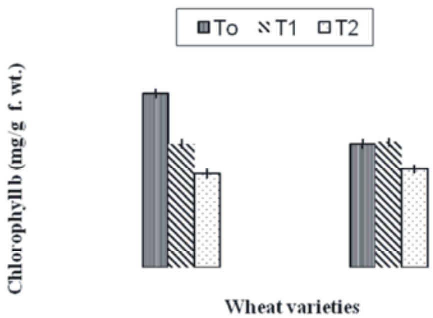

Fig. 8: Chlorophyll b contents of two wheat varieties after GB spray when plants were 47 days old [T1=50 and T2=100 mM respectively].

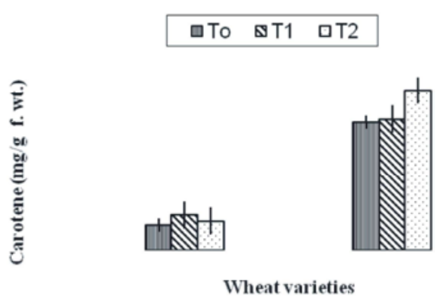

Fig. 9: Carotene contents of two wheat varieties after GB spray when plants were 47 days old [T1=50 and T2=100 mM respectively].

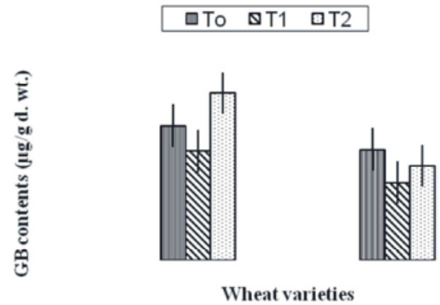

Fig. 10: GB concentration in two wheat varieties after exogenous application of GB when plants were 56 days old [T1=50 and T2=100 mM respectively].

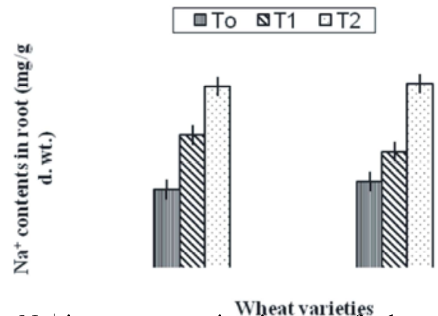

Fig. 11: Na^+ ion concentration in root of wheat varieties after GB exogenous application when plants were 60 days old [T1=50 and T2=100 mM respectively].

Fig. 12: Na^+ ion concentrations in shoot of wheat varieties after GB exogenous application, When plants were 60 days old [T1=50 and T2=100 mM respectively].

Photosynthetic Pigments: GB increased the chl. a and chl. b contents significantly (p=0.001) at 100 mM solution level. There is no significant variation in both varieties regarding chla andchl b (Fig.7, 8). However, GB showed no effect on carotene contents. There is highly significant (p=0.001) variation regarding carotene contents of both varieties. Chakwal-97 has high concentration of carotene than Sehar-2006 (Fig.9).

GB Concentration: Results showed that there was increase in GB concentration in both wheat varieties after GB foliar application at both the treatments (50 and 100 mM) as compared to control. However, GB concentration of 100 mM has significantly (p=0.001) more increasing effect on GB accumulation. There is highly significant variation in both varieties of wheat. Sehar-2006 had more GB accumulation as compared to Chakwal -97 (Fig.10).

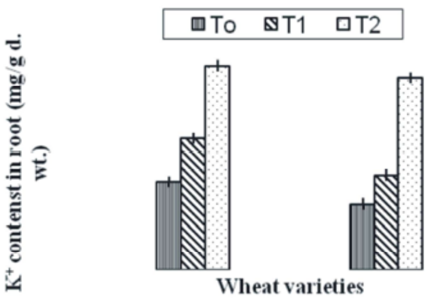

Fig. 13: K^+ ion concentrationin root of two wheat varieties after exposure to GB foliar application when plants were 60 days old [T1=50 and T2=100mM respectively].

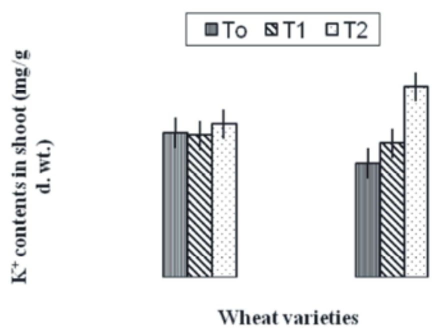

Fig. 14: K^+ ion concentration in shoot of wheat varieties after GB exogenous application when plants were 60 days old [T1=50, T2=100mM respectively].

Na^+ and K^+ ion Contents in Shoot: GB had increased the K^+ and Na^+ ion contents in shoot of both wheat varieties.

Na^+ and K^+ ion Contents in Root: GB had increased the Na^+ and K^+ ion contents in root of both wheat varieties.

DISCUSSION

Heavy metal stress stimulates the accumulation of GB, which provides protection in stress conditions [42]. It protects membrane integrity, scavenges reactive oxygen species, stabilizes proteins and promotes osmotic adjustment in plants [43, 44].

Foliar application of GB improves the growth vigor by increasing the shoot / root length and fresh /dry masses in both wheat varieties. In our experiment, GB has increased the shoot length of both wheat varieties, because GB has compensated the growth reduction in wheat plants. These results are parallel to another finding in which GB significantly increased the shoot length in wheat cultivars under drought condition. GB spray improved growth and yield in tobacco and wheat. Exogenous application of GB increased the root length of both wheat varieties. Similar results were observed by in wheat in which GB has also increased the root length [45, 46]. GB provides the tolerance to plants against heavy metal stress that cause oxidative damage to plants by increasing the antioxidant enzyme activities [47].

In present study after foliar application of GB, the fresh and dry weights of shoots and roots were increased significantly in both varieties. Similar results were obtained in an earlier study in which GB increased the weights of shoot and root in wheat cultivars [48]. In another study, GB increased all the shoot and root growth characteristics in wheat under abiotic stress [49]. GB increases the growth parameters and yield of plants because it provides tolerance to plants against harmful effects caused by heavy metal stress and minimizes the oxidative damage caused by heavy metals. GB caused increment in Chl. a and Chl. b in our study in both wheat varieties. Our findings are coherent with another result in which GB has positive effects on photosynthetic pigments [50]. Additionally, exogenously GB has increased chlorophyll concentration in maize under abiotic stress [51]. The current study findings suggest that exogenous application of GB increased the endogenous contents of GB in both the varieties of wheat. Similar results were observed in another study [52]. Consequently, higher level of GB caused enhanced contents of K^+ and Na^+ ions in root and shoot. Similar results with increased contents of K^+ and Na^+ due to higher applications of GB were observed in wheat [52].

CONCLUSION

Conclusively based on data, it is suggested that GB spray increased morphological parameters such as shoot and root length, biomass, photosynthetic pigments and Na^+, K^+ ion contents. Thus, it is stated that exogenous GB application isan applicable approach to deal effectively with heavy metal toxicity in wheat.

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