

EFFECT OF FOLIAR APPLICATION OF GLYCINE BETAINE AMELIORATED DROUGHT STRESS IN ENHANCING THE BIOMASS AND OTHER GROWTH-LINKED BIOCHEMICAL AND PHYSIOLOGICAL ATTRIBUTES IN WHEAT

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Abstract:

Drought stress causes significant losses in crop yield and other parameters of wheat crop. Glycine betaine (GB) is found in plants naturally and it helps the plant to cope with abiotic stress. In the present investigation, the effect of foliar application of Glycine betaine under drought stress was evaluated on wheat plants by adopting a completely randomized design (CRD). The effect of six treatments viz., Control, 80% field moisture capacity (FMC), 60% FMC, 5 mM GB, 80% FMC+5mM GB, and 60% FMC+5mM GB were investigated on the morphological, biochemical, and physiological attributes of wheat. Both 80 and 60% FMC showed a significant reduction in all these parameters but this decline was more pronounced at the former level of drought. The foliar application of GB significantly enhanced the shoot length, shoot fresh weight, chlorophyll, carotenoids, photosynthetic rate (P_n), transpiration rate (E), and stomatal conductance (g_s) by 24, 26, 10, 12, 16, 20, and 16%, respectively, as compared to control. Combined application of GB with drought stress also enhanced the plant height and shoot fresh weight by 28 and 41%, respectively, Chl a, b and carotenoids by 10, 10, and 12%, P_n , E , and g_s by 27, 47, and 35%, respectively, as compared to 60% FMC drought treatment level. So, based on these results, it is concluded that GB is an effective organic osmolyte that have promising potential to ameliorate drought stress and enhance the biomass and other growth-linked biochemical and physiological attributes.

Keywords: Drought stress, Morphology, Photosynthetic, Stomatal conductance.

Introduction

A major threat to ecosystems, agriculture, water resources, and human well-being worldwide is drought stress, a ubiquitous and growing environmental concern (Sakamoto & Murata, 2002; Zia *et al.*, 2021). Drought stress is characterized by extended periods of exceptionally low precipitation and water availability, and it can have significant effects on many facets of life on Earth (Abbass *et al.*, 2022). The frequency, intensity, and duration of drought events are growing as global climate patterns continue to change, amplifying their negative consequences on both natural and human systems (Weiskopf *et al.*, 2020).

Drought stress can reduce crop yields, delay plant growth, and even cause crop failure in agricultural systems (Hussain *et al.*, 2018). These errors interrupt food production, increase food scarcity, and boost food prices, resulting in an increase in poverty around the world. Drought stress greatly affects the amount of available water by reducing surface water bodies,

groundwater reserves, and soil moisture (Ahluwalia *et al.*, 2021). This scarcity has an influence on both industrial operations and human consumption, resulting in water rationing, water-borne infections, and severe economic repercussions in a variety of businesses (Saha *et al.*, 2022). Drought stress destabilizes ecosystems by altering plant diversity, reducing biodiversity, and compromising the health of wetlands, forests, and other natural habitats. These alterations have a negative impact on key functions (Hussain *et al.*, 2018; Ahmad *et al.*, 2022).

The world's food supply is threatened by declining water resources and challenged by rising dietary needs (Hanjra & Qureshi, 2010). The primary economic driver in Pakistan is agriculture, and among the major crops, rice dominates. Agriculture is the economic backbone of Pakistan. Pakistan's basic food is wheat, and as the country's population grows, so does the demand for food. A significant abiotic stress that can negatively affect wheat productivity is drought (Rehman *et al.*, 2015). Wheat plants are susceptible to

it at every stage of development, from germination to maturity (EL Sabagh *et al.*, 2021). The length and intensity of the drought, as well as the genetics of the wheat cultivar, will all have an impact on how severe the effect is (Tricker *et al.*, 2018; Lama *et al.*, 2023).

Glycine betaine (GB) is a naturally occurring chemical molecule found in a variety of plants (Ali *et al.*, 2020). It is an osmolyte, which implies that it protects cells from dehydration. Glycine betaine can also help plants resist additional stressors like heat, cold, and salinity (Sakamoto & Murata, 2002). Previous studies reported that GB can trigger the osmotic potential in plants by regulating the reactive oxygen species (ROS). Previous studies revealed that GB can ameliorate the drought effect on different crops but very few studies were present regarding wheat. So, the objective of this study is to check the drought mitigation potential of GB on wheat plant's attributes.

Material and methods

To investigate the effect of drought stress and foliar application of Glycine betaine (GB) on wheat crop, an experiment was conducted in Gujrat, Punjab, Pakistan. Wheat variety 2008 was grown in plastic pots having a diameter of 20 cm and a height of 25 cm. The pots were filled with 8 kg of soil. Five seeds were sown in each pot and on germination 2 plants were kept to check the effect of GB and drought.

Treatments: Following treatments were used to assess the effect of GB and drought stress on wheat plants.

T1= Control (100% Field Moisture Capacity (FMC))

T2= Wheat + 80% FMC

T3= Wheat+ 60 % FMC

T4= Wheat+ 5 mM Glycine betaine (GB)

T5= Wheat+ 80% FMC+5 mM GB

T6= Wheat+ 60% FMC+5 mM GB

Each pot was sprayed with 10 ml of 5mMGB spray. Pots were kept under greenhouse conditions to avoid rain and maintain the drought level.

Morphological Parameters: Morphological parameters were assessed after 25 days of treatment application. Shoot length, root length and their fresh

and dry weight was measured by physical means and electrical balance.

Biochemical Parameters: To determine the chlorophyll a, b, and carotenoids, similar methods were adopted as described by Arnone (1949) and Devis (1976).

Physiological Parameters: In physiological parameters, net photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (E) were measured with the help of an Infrared gas analyzer machine on full sunny days between 11:00 am to 2:00 pm.

Results and Discussion

Effect of Drought stress and GB on morphology of wheat: Drought stress significantly reduced the plant height of wheat plants by 27 and 48% respectively as compared to healthy control. On the other hand, foliar application of Glycine betaine (GB) increased the plant height by 24% as compared to the untreated healthy control. Moreover, foliar application of GB applied in combination with drought stress, plant height was significantly increased by 11 and 28%, respectively as compared to drought-bearing plants (Figure 1A).

Drought stress significantly reduced the shoot fresh weight of wheat plants at both drought levels, 80 and 60% FMC. However, this decline was more prominent at the 60% FMC level. On the other hand, the foliar application significantly enhanced the fresh weight by 26%, when applied alone. Furthermore, the combined application of GB with 80% FMC increased the fresh weight of wheat plants by 25%, and with 60% FMC, a prominent increase of 41% was recorded as compared to their alone drought level (Figure 1 B).

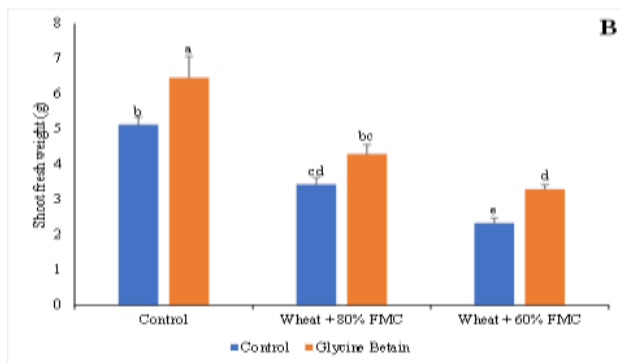
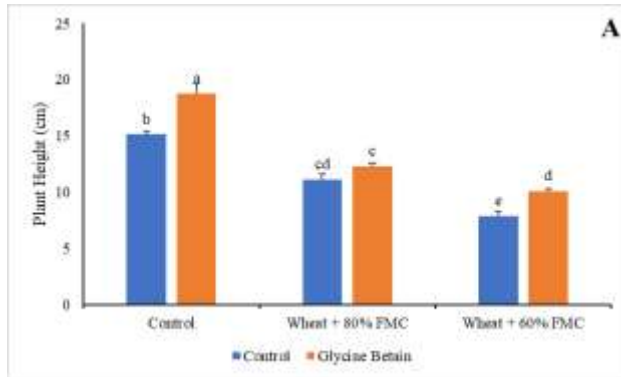


Figure 1: Effect of Glycine betaine on (A) Shoot length, (B) shoot fresh weight under drought stress.

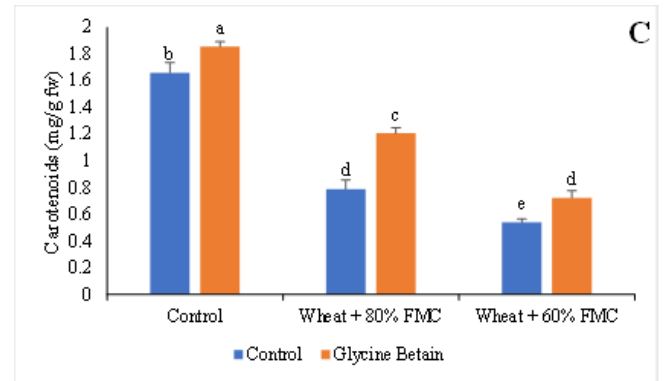
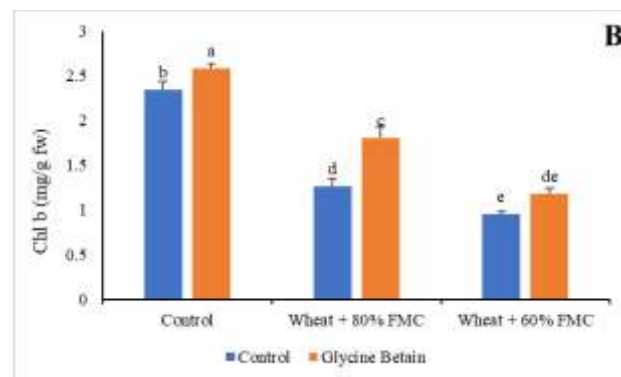
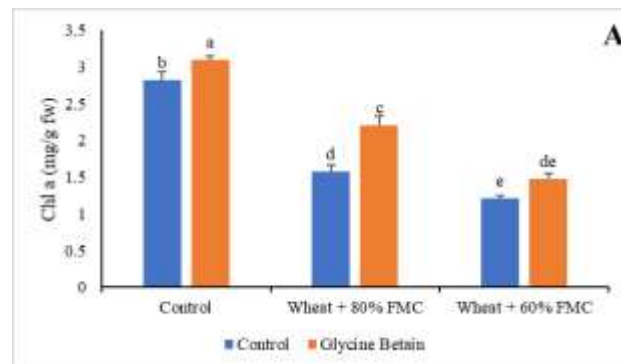


Figure 2: Effect of Glycine betaine on (A) Chlorophyll a, (B) Chlorophyll b and (c) Carotenoids of wheat under drought stress.

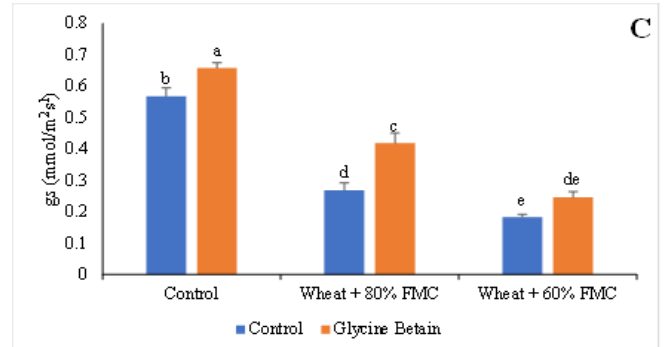
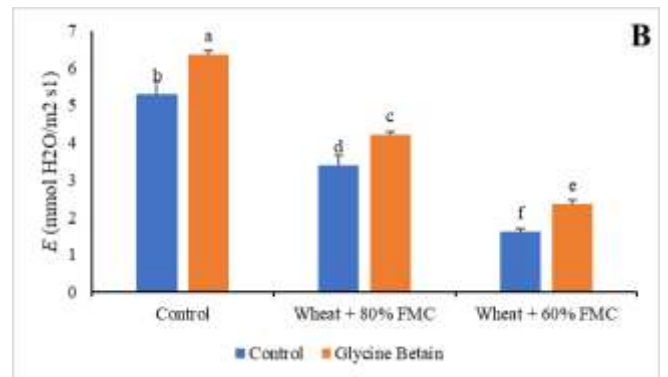
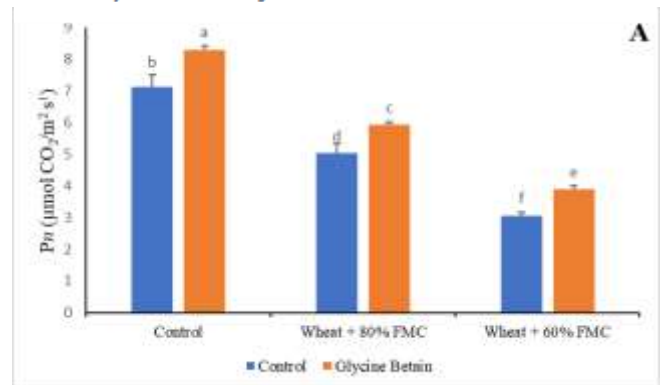


Figure 3: Effect of Glycine betaine on (A) Photosynthetic rate (Pn), (B) transpiration rate (E), and (c) stomatal conductance (gs) of wheat under drought stress.

Plant height reduces significantly with an increase in the severity of drought conditions. This reduction in the height may be a result of inhibition of cell elongation by severe drought stress (Yang *et al.*, 2021). Drought causes impaired germination and poor establishment of plants. Cell growth is one of the most important processes that is sensitive to drought. It reduces due to decreases in turgor pressure. Growth results from the production of daughter cells from meristematic cell division and the expansion of young cells (Osmolovskaya *et al.*, 2018). Under severe conditions of drought, cell division can be inhibited by interruption of water flow from the xylem to elongating cells (Wahab *et al.*, 2022). A very common and damaging effect of drought stress on crop plants is the reduction in fresh and dry weight (Zia *et al.*, 2021). Drought causes a decrease in the number of leaves per plant, individual leaf size, and longevity of leaves. Expansion in the leaf area depends upon the turgor pressure, temperature, and sufficient supply of nutrients for growth. Reduced leaf area under water stress is damaging because it leads to a reduction in nutrient uptake due to reduced transpiration (Fahad *et al.*, 2017).

Effect of Drought and Glycine betaine on wheat biochemicals and physiological attributes: Drought stress depicted the negative effect on photosynthetic pigments chlorophyll a, b and carotenoid and reduced them by 57, 59 and 67%, respectively, when plants were grown at 60% FMC, as compared to the control. On the other hand, GB showed statistically significant results and showed increased trend in Chl a, b, and carotenoid pigments. While, drought and GB combined effect also showed significant increase of 40% in Chl a, a 43% uplift in Chl b and 53% increase in carotenoid content (Figure 2 A-C).

In the present investigation drought stress significantly affected the gaseous exchange parameters and significantly reduced the photosynthetic rate (P_n) by 57 %, transpiration rate (E) by 70% and stomatal conductance (g_s) by 56%, as compared to control. Foliar application of GB enhanced the P_n and g_s by 16% and E by 20%, respectively, as compared to healthy control. On the other hand, combined application significantly mitigated the drought stress and enhanced the P_n , E , and g_s by 27, 47 and 56% against different FMC drought levels (Figure 3 A-C).

Drought stress reduces the pigments like chlorophyll a and chlorophyll b. Drought stress creates changes in the fraction of chlorophyll 'a' and 'b' and carotenoids (Shanthi *et al.*, 2023). A decrease in

chlorophyll content was testified in drought-stressed cotton. Drought stress decreases more chlorophyll content than chlorophyll b and total carotenoids (Zhuang *et al.*, 2020). As water is the most important element of photosynthetic activity and limiting of water impairs the photosynthetic rate. Drought stress greatly decreased stomatal conductance (g_s), and transpiration rate (E), and due to the decrease of these activities, photosynthesis also decreases (Seleiman *et al.*, 2021). Plants retort to stresses such as drought by decreasing leaf expansion and closing stomatal pores. In this way, they save the water, nutrients, and carbohydrates necessary for survival, which is helpful for sustaining optimal water status, photosynthesis, and plant growth (Dos Santos *et al.*, 2022).

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