



Asian Journal of Plant Sciences

ISSN 1682-3974

science
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Research Article

Impact of Glycine Betaine on Drought Tolerance of *Moringa oleifera* Plant Grown under Sandy Soil

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Abstract

Background and Objective: Glycine Betaine (GB) is one of the best powerful compatible solutes that save plants from abiotic stresses like drought. External treatment of GB is well known to regulate a myriad of physiological and biochemical processes in plants subjected to stress like drought. This research attempted to evaluate the effect of external treatment of Glycine Betaine (GB) on growth, photosynthetic pigments, endogenous Indole Acetic Acid (IAA), some osmoprotectants, yield quantity and quality, in the favour of total carbohydrates, proteins and antioxidant compounds in the yielded plants grown under normal as well as under drought-stressed conditions. **Materials and Methods:** A field experiment was carried out during two successive seasons in the Experimental Station of National Research Centre, Nubaria district, Beheira Governorate, Egypt. *Moringa oleifera* plants were treated with Glycine Betaine (GB) (10, 20 and 30 mM). **Results:** Water deficit significantly decreased *Moringa oleifera* growth and yield quantity and quality through decreasing photosynthetic pigments and IAA, accompanied by significant increases in some osmoprotectants and phenolics contents. Meanwhile, Different GB concentrations enhanced growth parameters and yield and its components via enhancing photosynthetic pigments, IAA, proline, TSS and free amino acids in *Moringa oleifera* plants under normal or drought stress conditions. In general, 20 mM GB was the most effective treatment in ameliorating the reduced effect of drought stress on *Moringa oleifera* plants. **Conclusion:** Finally, it can conclude that using different concentrations of GB as foliar treatment not only improved the growth and yield of *Moringa oleifera* plants under normal irrigation conditions but also, could ameliorate the decreased effect of drought on *Moringa oleifera* plants.

Key words: *Moringa oleifera* L., antioxidants activity, glycine betaine, osmoprotectants, drought, yield

Citation: Elhamid, E.M.A., M.S. Sadak, M.I. Ezzo and A.M. Abdalla, 2021. Impact of glycine betaine on drought tolerance of *Moringa oleifera* plant grown under sandy soil. Asian J. Plant Sci., 20: 578-589.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Moringa oleifera Lam. is a worldwide cultivated edible and medicinal tree. It is an Indian multipurpose plant cultivated in tropical and subtropical countries. It concerned as a very important economic plant due to its high nutritive values, so it named a miracle and drumstick tree. It has a multitude of medicinal and nutritive values because of the presence of vitamins, minerals, amino acids and antioxidants¹. Different parts of *Moringa oleifera* plants are edible as valuable vegetables such as roots, leaves, fruits and seeds. Its seeds are rich in oil, proteins and glucosinolates². In developing countries, *M. oleifera* is used as an alternative to imported food supplements to treat and combat malnutrition, especially among infants and nursing mothers, by its chemical constituents³. The chemical composition values confirmed that *M. oleifera* leaves powders are an excellent food source, justifying its direct use in human nutrition or the development of balanced diets for animal nutrition^{4,5}.

Drought, heat, salinity, etc. are various types of abiotic stress conditions that reduce plant growth and cause severe loss of crop yield due to different physiological, morphological and molecular level changes¹. The reduced effects of drought on plant growth and production resulted from reduced cell expansion, alterations in plant metabolism and decreases in the activities of different metabolic enzymes and decrease in respiration, translocation, ion uptake and levels of growth promoters⁶. Also, water deficiency affects adversely several biochemical and molecular processes, which results in stomatal closure, decrease in the rate of transpiration, pigment content, photosynthesis and thereby partial or full inhibition in growth and development. The induction of ABA synthesis is one of the fastest hormonal responses of plants to drought stress, thereby triggering ABA-inducible gene expression. This generally causes stomatal closure and reduces water loss via transpiration⁷. Moreover, water deficit resulted in the creation of Reactive Oxygen Species (ROS) which lead to leave damage and so, decrease crop growth and yield⁸.

To cope with such stress conditions, plants accumulate a wide variety of organic solutes called osmolytes. Osmolytes encompass amino acids and quaternary ammonium compounds like Glycine Betaine (GB), soluble sugars, sugar alcohols, proline, trehalose and polyhydric alcohols⁹. Osmolytes are accumulated in the cytoplasm as well as in chloroplasts in certain cases for osmotic adjustment under stress conditions. This enables the plants to absorb water and survive under stress¹⁰.

Glycine betaine is an amino acid derivative and considered one of the most effective compatible solutes

which protect plants from injury of abiotic stresses. The GB treatment enhances growth, survival and tolerance of various plants under different stress conditions via regulating different metabolic processes¹¹, improving net CO₂ assimilation rate, protecting the functional proteins, enzymes and lipids of photosynthetic apparatus and maintaining electron flow through thylakoid membranes and regulating photosynthetic machinery and ion homeostasis¹¹. Besides, it may act as an antitranspirant which allowed the plant to absorb more water for a long period and facilitates photosynthesis. Naturally produced GB can easily be collected as a relatively inexpensive natural by-product from high-producing plants such as sugar beets¹². This may make the application of GB an economically feasible approach to counteract the adverse effects of environmental stresses on crop productivity.

Thus, this investigation aimed to study the effect of foliar treatment of *Moringa oleifera* L. with Glycine betaine at different concentrations on growth, photosynthetic pigments, some osmoprotectants, yield quality and quantity.

MATERIALS AND METHODS

Plant material and growth conditions: *Moringa oleifera* L. plants were transplanted in the Experimental Station of National Research Centre, Nubaria district, Beheira Governorate, Egypt. This experiment was done during two successive seasons of 2017/2018 and 2018/2019. The soil of both experimental sites was newly reclaimed sandy soil where mechanical and chemical analysis is represented in Table 1 according to Chapman and Pratt¹³.

The experimental design of the experiment was a complete randomized block design with four replications. The main plots were devoted to the irrigation treatments, while Glycine Betaine (GB) treatments were randomly occupied the sub-plots. Each plant was fertilized with 40 g calcium superphosphate (15.5% P₂O₅) and 20 g potassium sulphate (48.0% K₂O) and 40 g urea (46.5% N) mixed with 500 g green manures (compost). Glycine betaine foliar treatment consisted of four levels of Glycine betaine namely 0.0 (control), 10, 20 and 30 mM considered as GB0, GB1, GB2 and GB3, respectively at 45 and 60 days after *Moringa oleifera* cutting. Drought stress including 100% Water Irrigation Requirement WIR (D0) and 75% WIR (D1). After second foliar treatment by 2 and 3 weeks, plant samples were taken. Estimation of some growth parameters as plant height (cm), fresh and dry weights of leaves and shoots (g), photosynthetic pigments of leaves, Indole Acetic Acid (IAA), Total Soluble Sugars (TSS), proline and free amino acids were measured. As the yield of *Moringa oleifera* here is foliage yield, yield and its

Table 1: Mechanical and chemical analysis of the experimental soil sites

Mechanical analysis									
Sand									
Course 2000-200 (μ %)		Fine 20-0 (μ %)		Soil texture 20-0 (μ %)			Clay<2 (μ %)		Soil texture
47.46		36.19		12.86			4.28		Sandy
Chemical analysis									
				Macro element (ppm)			Microelement (ppm)		
pH 1:2.5	EC (dS m ⁻¹)	CaCO ₃ (%)	OM (%)	N	P	K	Zn	Fe	Mn
7.60	0.13	1.5	0.06	52	12.0	75	0.14	1.4	0.3

components (foliage yield) as plant height (cm), stem diameter (cm), fresh and dry weight of shoots and leaves (g). Some nutritive contents of the yield plants were nitrogen, phosphorus, potassium, calcium and magnesium contents. In addition to some antioxidant compounds were phenolics, flavonoids, lycopene, B-carotene and DPPH activity.

Biochemical analysis: Chlorophyll a, chlorophyll b and carotenoids concentrations were estimated using the method of Lichtenthaler¹⁴. Indole acetic acid content was extracted and analyzed by the method of Larsen¹⁵. Total Soluble Sugar (TSS) was extracted¹⁶ and analyzed¹⁷. Free amino acids and proline were extracted¹⁸. The free amino acid was determined with the ninhydrin reagent method¹⁸. Proline was assayed¹⁹. Total carbohydrates amount were determined²⁰. The protein content was determined by the Micro Kjeldahl method³. Macro element contents of *Moringa oleifera* plants were determined³. Nitrogen and Phosphorus were determined using Spekol Spectrocolorimeter VEB Carl Zeiss. The Ca and K contents were estimated by using of flame photometer¹³. Total phenolic compounds were determined²¹. Total flavonoid contents were measured by the aluminium chloride colorimetric assay²². Lycopene and β -carotene analysis were determined²³. The free radical scavenging activity was determined²⁴ using the 1.1-diphenyl-2-picrylhydrazyl (DPPH) reagent.

Statistical analysis: The analysis of variance procedure of Complete Randomized block Design (CRD) with 5 replicates. Data were subjected to conventional methods of analysis according to the MSTAT-C²⁵ statistical analysis program. Means were compared by using Least Significant Difference (LSD) test at 5% of probability level. A combined analysis of the two growing seasons was carried out.

RESULTS

Growth parameters: Table 2 represented the effect of exogenous treatment of GB with different concentrations on *Moringa oleifera* plant under drought stress conditions. Data clearly show that 75% WIR caused significant decreases in growth parameters such as plant height, leaves, shoot and plant fresh and dry weight as compared with control plants (100% WIR). On the other hand, foliar treatment of Glycine betaine with different concentrations (10, 20 and 30 mM) caused marked and significant increases in various growth parameters of *Moringa oleifera* plant either at normal irrigation or drought-stressed conditions (Table 2). The most effective concentration of GB was 20 mM as it caused the highest increases in different growth criteria.

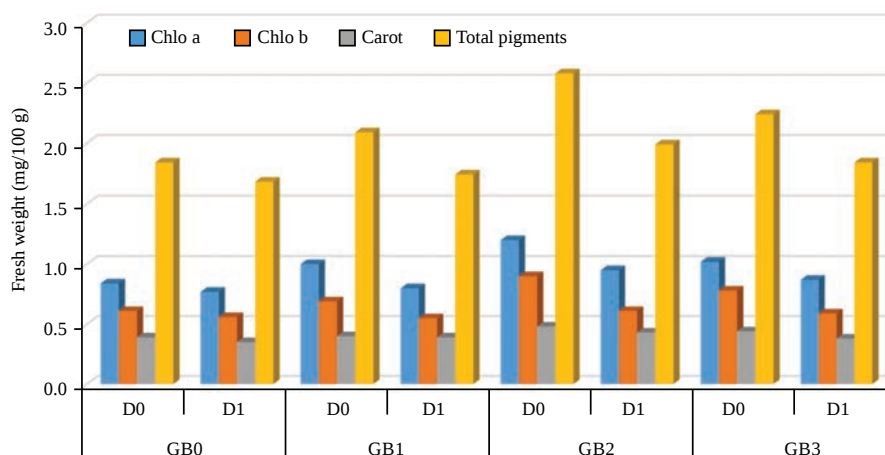
Photosynthetic pigments: Figure 1 showed the effect of different concentrations of GB foliar treatment on photosynthetic pigments constituents (Chlorophyll a, Chlorophyll b, carotenoids and total pigments) of *Moringa oleifera* leaves. Drought stress (75% WIR) significantly decreased photosynthetic pigments as compared with control plants (100% WIR). Meanwhile, different applied GB concentrations caused significant increases in all components of photosynthetic pigments in unstressed plants or drought-stressed plants compared with their corresponding untreated controls.

IAA content: Figure 2 showed the effect of foliar treatment of GB on *Moringa oleifera* plants under water deficiency. Drought stress (75 WIR) caused significant decreases in IAA contents in fresh *Moringa oleifera* leaves compared with that of control. In the contrast, GB treatments with different concentrations (0, 10, 20 and 30 mM) increased significantly endogenous IAA concentrations in unstressed *Moringa oleifera* plants as

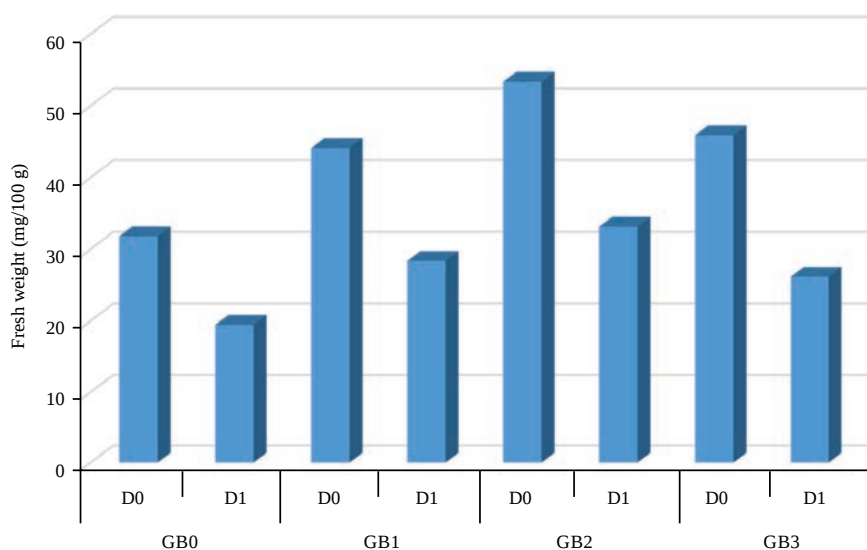
Table 2: Effect of glycine betaine on growth parameters of *Moringa oleifera* plants subjected to water deficit

GB	Water deficit	Plant height (cm)	Leaves fresh wt. (g)	Shoot fresh wt. (g)	Plant fresh wt. (g)	Leaves dry wt. (g)	Shoot dry wt. (g)	Plant dry wt. (g)
GB0	D0	110.7	15.4	42.0	57.4	3.9	12.4	16.3
	D1	84.0	14.0	31.2	45.2	3.0	9.3	12.3
GB1	D0	113.0	26.5	80.4	106.9	5.9	18.9	24.8
	D1	86.0	16.9	36.1	53.1	3.8	11.3	15.2
GB2	D0	128.3	47.4	83.0	130.5	10.5	19.0	29.6
	D1	93.0	27.0	49.4	76.3	6.9	13.5	20.4
GB3	D0	116.0	31.2	70.8	101.9	8.9	14.9	23.8
	D1	90.3	17.8	41.0	58.8	6.3	12.8	19.1
		9.65	1.132	7.658	6.958	0.674	2.654	1.325

GB0: 0, GB1: 10, GB2: 20, GB3: 30 mM, D0: 100%, D1: 75% WIR, data are means of two seasons, wt.: Weight

Fig. 1: Effect of glycine betaine on photosynthetic pigments (mg/100 g fresh weight) of *Moringa oleifera* plants subjected to water deficit

GB0: 0, GB1: 10, GB2: 20, GB3: 30 mM, D0: 100%, D1: 75% WIR, LSD at 5%: Chlo a: 0.126, Chlo b: 0.242, Carotenoids: 0.103, Total pigments: 0.254

Fig. 2: Effect of glycine betaine on IAA content (µg/100 g fresh weight) of *Moringa oleifera* plants subjected to water deficit

GB0: 0, GB1: 10, GB2: 20, GB3: 30 mM, D0: 100%, D1: 75% WIR, LSD at 5%: 4.523

well as in drought-stressed plants by increasing GB concentrations relative to their corresponding controls. The 20 mM GB had the highest beneficial effect in ameliorating the harmful effect of drought stress on IAA either under moderate or severe drought stress relative to corresponding controls.

Compatible solutes (total soluble sugars and proline) and free amino acids: Figure 3 clearly show that water deficiency (75% WIR) caused significant increases in Total Soluble Sugars (TSS) content of *Moringa oleifera* leaves compared with control plants (100% WIR). Moreover, all the used concentrations of GB caused more increases in TSS compared with untreated control plants under normal irrigated plants

and drought-stressed conditions. Data show that these increases were significant with different concentrations. The most effective concentration was 20 mM in increasing TSS contents of *Moringa oleifera* leaves.

Figure 4 clearly show that water deficiency (75% WIR) caused significant increases in proline and free amino acids contents of *Moringa oleifera* leaves compared with control plants (100% WIR). Moreover, all the used concentrations of GB caused more increases in different compatible solutes (as proline) as well as free amino acids contents compared with untreated control plants under normal irrigated plants and drought-stressed conditions. Data show that these increases were significant with different concentrations.

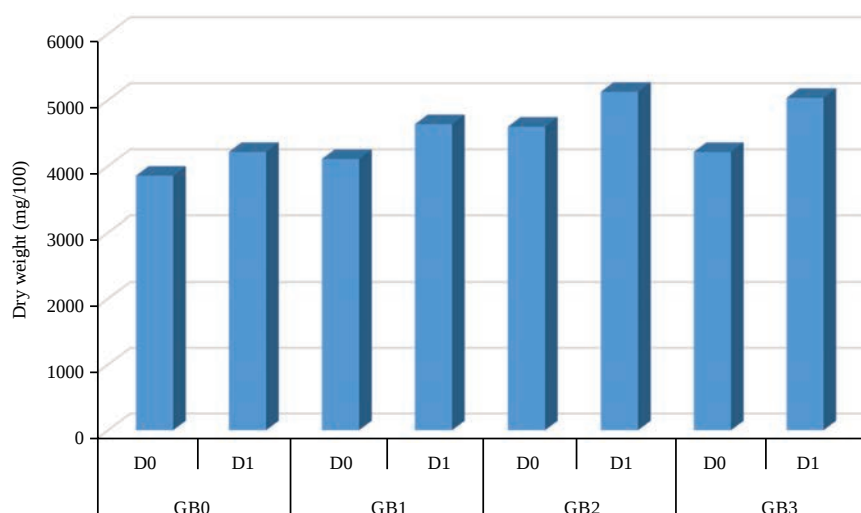


Fig. 3: Effect of glycine betaine TSS (mg/100 g fresh weight) of *Moringa oleifera* plants subjected to water deficit
GB0: 0, GB1: 10, GB2: 20, GB3: 30 mM, D0: 100%, D1: 75% WIR, LSD at 5% for TSS: 4.652

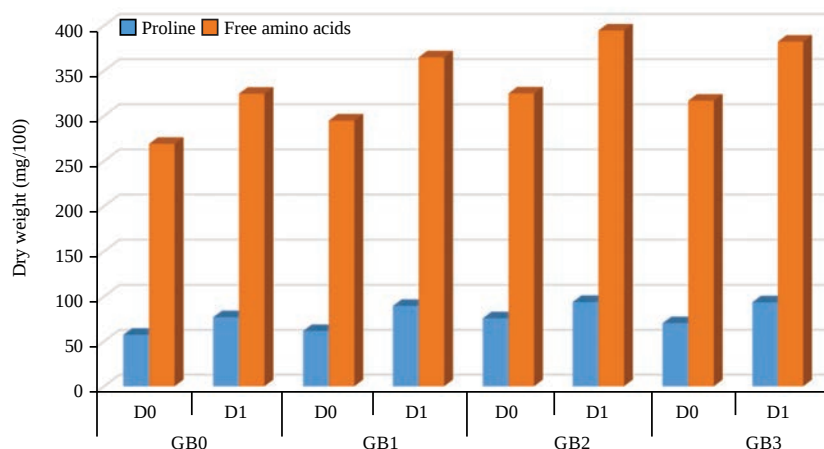


Fig. 4: Effect of glycine betaine on proline and free amino acids (mg/100 g fresh weight) of *Moringa oleifera* plants subjected to water deficit
GB0: 0, GB1: 10, GB2: 20, GB3: 30 mM, D0: 100%, D1: 75% WIR, LSD at 5%; proline: 3.625, total free amino acids: 26.659

The most effective concentration was 20 mM in increasing proline and free amino acids contents of *Moringa oleifera* leaves.

Foliage yield and its components: Data presented in Table 3 shows the effect of foliar treatments of different concentrations of GB on foliage yield of *Moringa oleifera* plant grown under drought stress. Subjecting *Moringa oleifera* plant to drought stress (75% WIR) caused significant decreases in various tested foliage yield parameters like plant height, stem circumference, leaves, shoot plant fresh and dry weights as compared with control plants. On the other hand, treatments of *Moringa oleifera* plants with Glycine betaine could improve different yield components as compared with untreated controls either at normal irrigation or drought-stressed conditions. GB with 20 mM was the most effective concentrations on different yield parameters of *Moringa oleifera* plants in Table 3.

Changes in carbohydrate and protein percentages in yielded grains: Data in Fig. 5 showed that water deficit (75% WIR) decreased significantly carbohydrates and protein

percentages of *Moringa oleifera* yielded plants as compared with those plants grown under normal irrigation conditions. Data also show significant increases in carbohydrates and protein % of *Moringa oleifera* yielded plants treated with different concentrations of GB. Foliar treatment of 20 mM GB was the most effective treatment as compared with the untreated plant and the other treatments under both normal and stressed conditions.

Changes in macronutrient and micronutrient contents in yielded grains: Concerning the effect of Glycine Betaine (GB) treatments on nitrogen, phosphorus calcium and magnesium contents of yielded *Moringa oleifera* plant under drought stress conditions. Data presented in Fig. 6 revealed that water deficiency decreased significantly yielded leaves contents of nitrogen, phosphorus, calcium and magnesium as compared with control plants (those plants grown under normal irrigation conditions). Meanwhile, foliar spraying of wheat plants with different concentrations of GB acid stimulated nitrogen, phosphorus, calcium and magnesium contents of the yielded *Moringa oleifera* plants as compared with control plant. Glycine betaine with 20 mM was the most effective

Table 3: Effect of glycine betaine on foliage yield and its components of *Moringa oleifera* plants subjected to water deficit

GB	Water deficit	Plant height (cm)	Stem diameter (cm)	Leaves fresh wt. (g)	Shoot fresh wt. (g)	Plant wt. (g)	Leaves dry wt. (g)	Stem dry wt. (g)	Plant wt. (g)
GB0	D0	260.6	11.7	103.5	198.7	302.2	20.8	73.0	93.8
	D1	198.7	10.0	38.0	183.5	221.6	8.2	40.0	48.2
GB1	D0	438.1	13.7	142.2	438.1	580.3	39.9	122.0	161.9
	D1	263.0	12.2	59.8	263.0	322.8	15.6	48.2	63.8
GB2	GB0	598.2	14.7	199.9	598.2	798.1	43.5	131.0	174.5
	GB1	438.1	13.0	91.2	366.5	457.6	20.6	79.7	100.3
	GB2	366.5	13.0	137.1	483.8	620.9	29.5	90.0	119.5
	GB3	288.4	13.0	58.7	288.4	347.1	12.5	73.4	85.9
LSD at 5%		25.624	1.514	14.625	32.654	36.652	3.214	5.514	7.658

GB0: 0, GB1: 10, GB2: 20, GB3: 30 mM, D0: 100%, D1: 75% WIR, data are means of two seasons, wt.: Weight

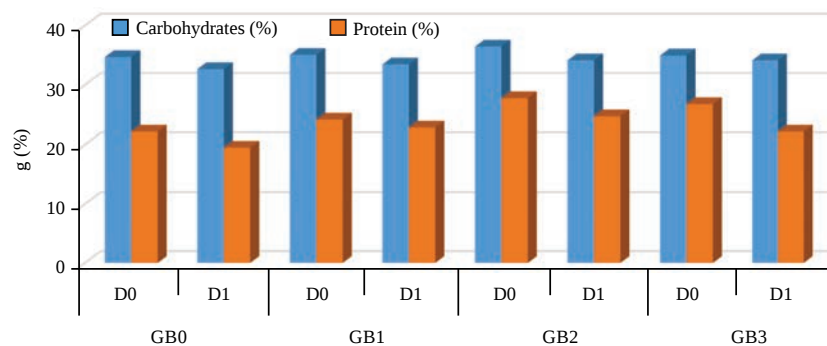


Fig. 5: Effect of glycine betaine on carbohydrate and protein percentages of *Moringa oleifera* yielded plants subjected to water deficit

GB0: 0, GB1: 10, GB2: 20, GB3: 30 mM, D0: 100%, D1: 75% WIR, LSD at 5% for carbohydrates (%): 0.925, protein (%): 0.345

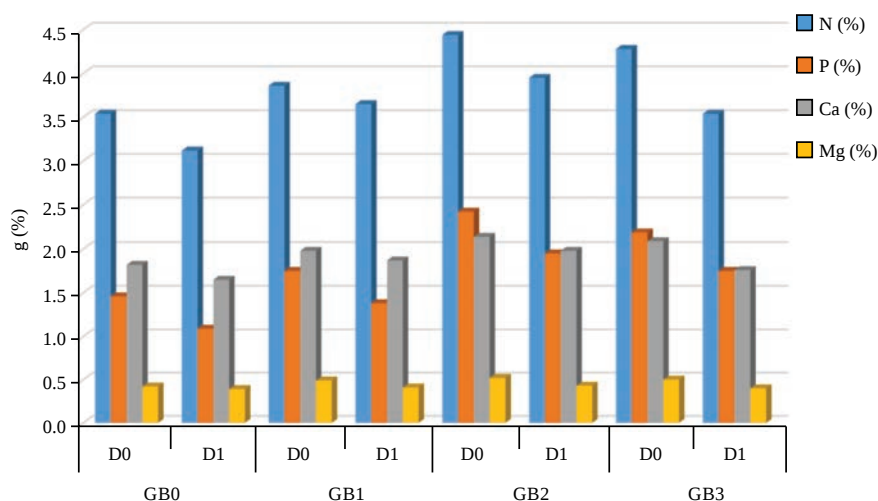


Fig. 6: Effect of glycine betaine on element percentages of *Moringa oleifera* yielded plants subjected to water deficit

GB0: 0, GB1: 10, GB2: 20, GB3: 30 mM, D0: 100%, D1: 75% WIR, LSD at 5%, N (%): 0.345, P (%): 0.452, Ca: 0.452 and Mg (%): 0.031

treatment as it gave the highest contents of different studied macro elements under normal and stressed conditions.

Phenolics, flavonoids and non-photosynthetic pigments (Lycopene, B-carotene) in the yielded *Moringa oleifera* plants:

Concerning phenolics, flavonoids and non-photosynthetic pigments as Lycopene and B-carotene contents in the yielded leaves of *Moringa oleifera* plants in response to water deficit and GB foliar treatment, Table 4 clearly showed that subjecting *Moringa oleifera* plants to drought stress significantly increased phenolics and flavonoids contents of the yielded leaves of *Moringa oleifera* plants. Meanwhile, significantly decreased lycopene and B-carotene content of the yielded leaves as compared with the control plant. Meanwhile, foliar treatment of *Moringa oleifera* plants with GB different concentrations caused marked and significant increases in phenolics, flavonoids, lycopene and B-carotene contents of the yielded leaves of *Moringa oleifera* plants (Table 4). Data showed that 20 mM was the most effective treatment for increasing the above-mentioned parameters under normal and drought-stressed conditions.

Antioxidant activity in yielded grains: Data in Table 4 showed that skipping irrigation of *Moringa oleifera* plant two times caused significant increases in antioxidant activity (as DPPH-free radical scavenging capacity) of *Moringa oleifera* leaves as compared with those plants irrigated normally. Data also showed that foliar treatment with GB (10, 20 and 30 mM) caused significant increases in antioxidant activity as compared with their corresponding

Table 4: Effect of glycine betaine on phenolics, flavonoids lycopene, B-carotene (mg/100 g) and DPPH (%) of *Moringa oleifera* yielded plants subjected to water deficit

Water						
GB (mM)	deficit	Phenolics	Flavonoids	Lycopene	B-carotene	DPPH
GB0	GB0	11.55	3.16	0.270	0.379	57.87
	GB1	13.65	3.83	0.226	0.256	61.25
GB1	GB2	13.35	3.88	0.308	0.486	60.80
	GB3	16.43	4.16	0.291	0.319	63.51
GB2	GB0	14.75	4.17	0.377	0.578	65.73
	GB1	17.26	4.79	0.317	0.337	66.71
GB3	GB2	13.17	3.76	0.364	0.460	65.73
	GB3	16.40	4.18	0.283	0.286	65.15
LSD at 5%		1.352	0.426	0.0215	0.0175	3.6544

GB0: 0, GB1: 10, GB2: 20, GB3: 30 mM, D0: 100%, D1: 75% WIR

control plants under normal and stressed conditions. Higher content of the antioxidant activity was obtained with 20 mM of GB application.

DISCUSSION

Water deficiency is one of the main factors affecting plant growth and yield loss all over the world. Drought stress is projected to decrease the average yields of major crops by more than²⁶ 50%. It was stated that plants modulate their physio-biochemical processes features according to the internal or external climate to survive under such harsh conditions²⁷. In this investigation, different growth parameters reduced under water deficit conditions (Table 2), this was following Bhardwaj²⁸ on wheat plant and Shehzadi²⁹ on oat plant. Sadak³⁰ stated that water stress-decreased growth is linked to the interior status of the plant concerning specifically photosynthesis, nutrients, hormones, antioxidants, primary and secondary metabolites, respiration etc. Moreover, these

decreases could be attributed to alterations in various metabolic processes such as decreased cell elongation and division, cell turgor and finally cell death³¹. On the other hand, Glycine betaine external treatment enhanced the growth of the *Moringa oleifera* plant under normal conditions as well as, improved plant tolerance to drought stress by increasing growth parameters. This promotive effect of GB could counteract the reduced effects of drought by enhancing growth vigour of the shoot, enhancing pigments constituents, increasing osmoprotectants contents, keeping out the polysaccharides concentration and/or stabilization of essential proteins³².

The reduced effect of drought stress on different photosynthetic pigments of *Moringa oleifera* plants are presented in Fig. 1. These decreases could be attributed to the disturbances in different physiological processes resulted from lipid chloroplast oxidation thus alters the structure of proteins and pigments³³. These data are in agreement with Qu³⁴ on oil tea, Bhardwaj²⁸ on wheat Sadak and Ramadan³⁵ on white lupine plant. Treatment of GB was effective in the alleviation of the harmful effects of water deficit conditions on the photosynthetic capacity of plants possibly due to its role in preventing photo-inhibition¹², protection of Rubisco enzyme and lipids of the photosynthetic apparatus and maintaining electron flow through thylakoid membranes thereby maintaining photosynthetic efficiency³⁶.

Water deficiency caused significant decreases in IAA contents in fresh *Moringa oleifera* leaves than that of control (Fig. 2). These decreases might be attributed to the increase in IAA oxidase activity which increases IAA destruction³⁷. In contrast, GB treatments with different concentrations significantly increased IAA concentrations in unstressed plants as well as in drought-stressed plants. Aldesuquy³⁸ mentioned that exogenous spray with GB may increase the drought tolerance by the acceleration of growth promoters (IAA, GA3 and cytokinins) and at the same time reduced accumulation of inhibitor represented by ABA in flag leaves of wheat plants. Underwater deficiency stress, plants accumulate greater amounts of compatible organic solutes and free amino acids that shield them from stress via stabilizing of membranes, tertiary structures of enzymes and proteins⁸. Figure 3 and 4 states that drought stress caused significant increases in TSS, proline and free amino acids contents of *Moringa oleifera* leaves relative to control plants. These results are consistent with Zafar³⁹, Liagat³² and Bhardwaj²⁸ and Rasheed⁴⁰ they stated that water stress caused accumulation of TSS, proline and free amino acids in flax, canola, *Moringa oleifera* and sunflower leaves. Regarding TSS, these accumulations of TSS could increase the resistance of the plant to drought stress⁴¹.

Regarding the increases in proline contents, these increases could be attributed to decreased proline oxidase activity, proline catabolizing enzymes as mentioned by Debnath⁴². Moreover, proline is considered as a carbon and nitrogen source for rapid recovery from stress and acting as a stabilizer for membranes and some macromolecules and also as a free radical scavenger⁹. Moreover, GB treatments caused more increases in TSS, proline and free amino acids concentrations in *Moringa oleifera* leaves under drought stress as well as in unstressed plants. These accumulations under drought stress and GB treatment are consistent with the early results of Hasanuzzaman⁴³ and Tisarum⁴⁴. Glycine Betaine GB is one of the most extensively studied compatible solutes that not only acts as an osmoregulatory but also stabilizes the structures and activities of enzymes and protein complexes and maintains the integrity of membranes against the damaging effects of water deficiency in different plant species⁴⁵.

Water deficiency stress caused significant decreases in foliage yield and its components of the *Moringa oleifera* plant (Table 3). These decreases due to the decreases in photosynthetic pigments⁴⁶ and diminished activities of Calvin cycle enzymes. Glycine Betaine (GB) promotes plant growth and yield under normal or stress conditions due to its osmoprotective effect on photosynthetic machinery and regulation of ion homeostasis¹² as well as improving CO₂ assimilation in plants under drought stress and because of its role in biosynthesis and transport of hormones like cytokinins that may have a role in the transport of photoassimilates⁴⁷.

Water deficit caused significant decreases in total carbohydrate and protein contents of the yielded leaves of *Moringa oleifera* (Fig. 5). This decreases in carbohydrates and protein contents are mainly due to the low water supply during plant life reduced activities of many enzymes thus leading to disturbance in metabolic activities mainly photosynthetic pigments which resulted in altered assimilates translocation. Drought stress reduces the concentration of carotenoids, chlorophyll a, chlorophyll b, total chlorophylls in Canola plant leaves and the chlorophyll a and b ratios were increased⁴⁸. On the other hand, the stimulating effect of GB treatments on carbohydrate and protein contents might be due to the increases in photosynthetic pigments (Fig. 1). As well, these increases in carbohydrate contents might be due to the increased photosynthetic output⁴⁹.

Nitrogen, phosphorus, potassium, calcium and magnesium contents of *Moringa oleifera* plants decreased significantly under water deficit (75% WIR) compared with control plants (100% WIR). These reduced effects of drought on element contents are in good agreement with the obtained earlier by several authors as Peuke and Rennenberg⁵⁰

on beech and Arjenaki⁵¹ on wheat plant. The decreases in K and Mg caused decreases in photosynthetic pigments contents and thus utilization of fixed carbon⁵². Calcium is an essential component of membranes and cell wall that has large functions in metabolic processes such as signalling pathway and stress response. The Ca is the most effective divalent and stabilizes cell membrane under abiotic stress via mediating membrane-associated such as the reduction of ion leakage, uptake of ions and amino acids and maintenance of the configuration of enzyme binding-sites in cells⁵³. On the other hand, GB foliar treatment increased element contents of *Moringa oleifera* plants either at normal irrigated plants or at drought-stressed plants (Fig. 6). The increases in leaf N, P, K, Ca and Mg of *Moringa oleifera* plants under drought is likely owing to the promoting effect of GB on the accumulation and absorption of these elements.

In response to water deficiency, different physiological and biochemical mechanisms have been developed in plants to alleviate stress. Drought stress-induced disturbances in the metabolic process leading to the increase in the synthesis of phenolic compounds. Phenolic compounds play an important role as antioxidants in scavenging free radicals arising from their high reactivity as hydrogen or electron donors, to stabilize and delocalize the unpaired electron (chain-breaking function) and from their ability to chelated transition metal ions⁵⁴. These metabolites may participate in Reactive Oxygen Species (ROS) scavenging mainly through the antioxidative enzymes utilizing polyphenols as co-substrates⁵⁵. Regarding flavonoids, they are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups. Flavonoids have been found to act as antioxidant molecules against drought stress, enhancing the oxidative tolerance of many plants subjected to water shortage. GB application increased the concentration of total flavonols during the drought period⁵⁶. The increased contents of flavonoid under stress conditions may reflect some kind of defence against stress conditions (i.e., oxidative burden).

Non-photosynthetic pigments such as lycopene, an important phytochemical are well known for their significant potential to serve as a remarkable antioxidant molecule⁵⁷. Free radicals which cause oxidative damage in the body have proven to play a significant role in the emergence of various chronic diseases including cancer, ageing and cardiovascular disorders⁵⁸. Lycopene as a precursor of β -carotene is fat-soluble carotenoids that exhibit two-fold higher antioxidant activity than β -carotene. The obtained data of the changes in nutritional values and antioxidant potentials of the yielded

Moringa oleifera plant as lycopene and β -carotene contents and antioxidant activity as DPPH% in response to GB foliar treatments and drought stress are presented in Table 4. Lycopene and β -carotene are generally known as powerful natural antioxidants that act as the most efficient singlet oxygen quenchers *in vitro* among common carotenoids. Osmolytes appear to have multiple functions during stress such as osmotic adjustment and scavenging of Reactive Oxygen Species (ROS). Thus, the generation of ROS and osmolytes accumulation is linked together¹¹. It has been reported that exogenously applied osmolytes increased the contents of antioxidant compounds under stress conditions. The increases in these metabolites due to osmolytes was found to be negatively associated with leaf MDA contents, thus showing the role of these osmolytes in plant oxidative defence mechanism by increasing the accumulation of these antioxidant secondary metabolites under stress conditions. Such effects of exogenously applied osmolytes in increasing the contents of these compounds may be due to the reason that these compatible solutes act as a regulatory or signalling molecule to activate multiple physiological and biochemical processes as well as plant adaptation processes under stress conditions^{13,58}.

CONCLUSION

From the present study, it could be concluded that different GB concentrations can enhance the growth and productivity of *Moringa oleifera* through enhancing photosynthetic pigments, increased IAA, proline, TSS and free amino acids either normally or under drought stress conditions.

SIGNIFICANCE STATEMENT

This study discovered the effective role of Glycine betaine that can be beneficial for alleviating the reduced effect of drought stress on plant growth and productivity via improving different biochemical and physiological metabolic processes. So, this study will help the researchers to uncover the critical areas of the physiological effect of GB on the *Moringa oleifera* plant under the effect of water deficiency that many researchers were not able to explore.

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