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Effect of glycinebeatine on wheat (*Triticum aestivum* L) under sandy soil conditions

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Abstract

Background: This study aimed to investigate the role of glycinebetaine in inducing the tolerance of two wheat cultivars to sandy soil conditions. Grains of Sids 12 and Misr 3 cultivars were soaked with glycinebetaine (GB) at 5 or 10 mM for 12 hours and dried at room temperature before sowing during two winter seasons 2019/2020 and 2020/2021. Results showed that plant growth of Misr 3 cultivar under sandy soil conditions was more adaptable than those of Sids 12 cultivar. In both cultivars, GB at 5 or 10 mM caused significant increases in most parameters measured in this study. Conclusions: Under sandy soil conditions, the quantity and quality of wheat cultivars have been enhanced using GB at 5 and 10 mM. 10 mM GB is considered the optimum treatment.

1. Introduction

Wheat (*Triticum aestivum* L) is regarded a strategic food crop around the world. Due to the restricted regions of the Nile Valley and rivalry with other crops, Egypt's wheat grown area needs be increased in new territory to close the gap between production and consumption [1].

Plants growing in sandy soil encounter a variety of environmental stresses, i.e. deficiency of water and nutrient, salinity of water and soil, high irradiances and temperature changes. In this regard, significant efforts must be made to increase plant tolerance to such conditions by selecting tolerant genotypes, employing the best cultural practices, and/or treating seeds (before sowing) or plants (at various stages of growth) with osmolytes, which play a crucial role in assisting plants in partially overcoming unfavorable conditions and avoiding negative effects on yield quantity and quality.

Plant growth and development are influenced by a variety of environmental conditions. Any change in these parameters has a negative impact on plant physiological and biochemical processes such as reduction of osmotic nutrients, denaturation of enzymes, and accumulation of Reactive Oxygen Species (ROS) and membrane dysfunction [2].

The accumulation of osmolytes, which plays a crucial role in improving plant tolerance, is one of the several plant responses to changes in environmental conditions [3, 4]. Under control and stress conditions, many types of osmolytes such as glycinebetaine (GB), trehalose, and proline were critical and increased physiological attributes [5].

There was a variability in ability of GB synthesize among plant species. Some plants, such as sugar beet, spinach, wheat, sorghum, corn and barley accumulate relatively moderate levels of GB in chloroplasts to improve their tolerance [6]. On the other hand, some plants do not effectively synthesize this compound such as *Arabidopsis* and tobacco, [7-9]. However, the naturally synthesized GB is not enough to ameliorate various environmental stresses [7]. So, GB is being used successfully to alleviate stress in GB-accumulating and non-accumulating plants. [10-11]. According to Makela *et al*. [12] GB penetrates rapidly to plant leaves and immediately moves to plant roots and meristems to protect plant parts against stress damages. The efficacy of GB application is dependent upon the species, plant developmental status, application level, and number of applications, among other factors [11].

GB acts as growth promoter and organic osmolyte that applied through a number of different ways (seed soaking, rooting medium, or as a foliar spray) and actively used in regulating the normal processes of plants [5]. Applications of GB on different plant species grown under normal or stressed conditions play an effective role in maintaining the osmotic adjustment [11], protecting the photosynthetic mechanism [8], membrane structure and chloroplasts from damage [13], stabilizing structures and functions of certain macromolecules [14-15]. Moreover, GB Applications protect quaternary structures of complex proteins and enzyme activity such as rubisco [16], reduce oxidation of membrane lipid [17], enhance antioxidative defense systems [18-19]. It protects electron transport in mitochondria [3], and normalizes the expression of genes [20].

GB regulates the growth and yield of plants under normal or stress conditions because of its osmoprotective impact on photosynthetic apparatus and maintaining of ion homeostasis [21-22] as well as enhancing CO_2 assimilation in plants [17] and may be due to its role in biosynthesis and transport of hormones like cytokinins that play a role in the transport of photo assimilates [23].

Seed priming is the cheapest approach and has been proved to be a successful approach to enhance plant growth and productivity [24]. Moreover, seed priming is more practical for agricultural activities.

This work aimed to investigate the role of glycinebetaine in inducing the tolerance of two wheat cultivars to sandy soil conditions.

2. Methods

Two field experiments were conducted at the experimental station of the National Research Centre, Nubaria district, El-Behera Governorate-Egypt, during two winter seasons 2019/2020 and 2020/2021. Two wheat cultivars (Sids 12 and Misr 3) were obtained from the Agricultural Research Centre, Giza, Egypt. Grains of Sids 12 and Misr 3 cultivars were soaked with GB at 5 or 10 mM for 12 hours and dried at room temperature before sowing. Then, wheat grains were sown in rows 3.5 meters long with 20cm distance between rows, Plot area was 10.5 m^2 (3.0 m in width and 3.5 m in length). The soil texture was sandy soil with the following characters: sand 85.3%, silt 10.7%, clay 4%, pH 7.84, CaCO3, 1.0%, EC 3.95 dsm^{-1} and the available total N, and K were 8.0, 3.0 and 19.8 ppm, respectively at 30 cm depth according to the method reported by Chapman and Pratt [25].

The recommended agricultural practices of growing wheat grain were applied according to the Agricultural Research Centre, Giza, Egypt. Moreover, the experiments were conducted and designed in a split plot experimental design with four replicates.

Plant samples were collected after 60 days from sowing for measurements of some growth parameters (shoot height, number of branches and leaves/plant as well as shoot fresh and dry weight). Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in fresh leaf tissues were determined as method described by Moran [26].

At harvest, the following parameters were documented on random samples of plants in each treatment: spike length; spike numbers; spike weight; grains number and weight/spike; 100 grains weight; straw yield and grains yield/fadan (Kg). The collected grains were used for determination of soluble sugars, total carbohydrate, and proline contents. Total carbohydrate and soluble sugar content were determined using the colorimetric method described by Dubois *et al.* [27]. Polysaccharides were calculating by subtracting soluble sugar from total carbohydrate. Proline was estimated according to Bates *et al*. [28].

Analysis of variance and differences among means were determined using least significant differences (L.S.D) at 5% level of probability using ASSISSTAT Software according to Silva and Azevedo [29].

3. RESULTS

3.1. Cultivars variation

Data recorded in Table 1 show that vegetative growth parameters of wheat plants belong to Misr 3 cultivar was surpassed than those of Sids 12 cultivar. Significant variation appeared in shoot height, fresh and dry weight of shoot. It is noted that shoot dry weight of Misr 3 cultivar was approximately twice the shoot dry weight of Sids 13 cultivar under sandy soil conditions. Data recorded in Table 2 show that total photosynthetic pigments and its components were significantly higher in Misr 3 cultivar than those of Sids 12 cultivar. Total photosynthetic pigments of Misr 3 cultivar were significantly higher than that of Sids 12 cultivar by 1.25. Data recorded in Table 3 show that grains yield and yield components of Misr 3 cultivar were higher than that of Sids 12 cultivar under sandy soil conditions. Grains yield of Misr 3 cultivar was higher than that of Sids 13 cultivar by 1.08. Data recorded in Table 4 indicate that yielded grain of Misr 3 cultivar was characterized by significant increases in soluble sugars, polysaccharides, total carbohydrates and proline contents than that of Sids 12 cultivar.

3.2. Glycinebetaine application

Data recorded in Table 1 show that GB treatments (5 and 10 mM) increased vegetative growth parameters by increasing its concentration. 10 mM GB caused significant increases in vegetative growth parameters under investigation. Data recorded in Table 2 show that GB treatments (5 and 10 mM) significantly increased total photosynthetic pigments and its components. Since, 5 mM GB significantly increased total photosynthetic pigments by 9.83% and 10 mM GB significantly increased total photosynthetic pigments by 30.84%. Data recorded in Table 3 show that GB treatments (5 and 10 mM) significantly increased grains yield and its components. 5 mM GB increased grain yield by 14.52% and 10 mM GB increased grain yield by 19.45% relative to control. Data recorded in Table 4 show that GB treatments (5 and 10 mM) significantly increased the investigated biochemical parameters relative to control.

3.3. Interaction between two wheat cultivars and GB treatments

It was noted that GB treatments increased vegetative growth parameters in both cultivars (Table 1). 10 mM GB was the most optimum treatment that caused the highest increases in all vegetative growth parameters under investigation. Moreover, it was noted that both applied GB treatments significantly increased all components of photosynthetic pigments relative to corresponding controls accompanied by non-significant decreases in A/B ratio (Table 2). 5 mM GB increased total photosynthetic pigments of

Sids 12 by 8.97% and Misr 3 by 10.53 % whereas, 10 mM GB increased total photosynthetic pigments of Sids 12 by 18.59% and Misr 3 by 41.39%.

Table 1: Glycinebetaine effects on growth characters of two wheat cultivars under sandy soil conditions

Cultivars	GB (mM)	Shoot height (cm)	Branches number/ plant	Leaves number/ plant	Shoot fresh weight(g)	Shoot dry weight (g)
Sids 12	0	71.56	1.44	6.45	6.26	2.7
Misr 3	0	88.44	1.56	7.33	11.7	4.00
LSD at 5% level		3.63	0.4195	1.235	1.19	0.362
	0	72.83	1.17	5.83	8.10	2.69
	5	81.50	1.33	6.17	8.26	2.96
	10	85.67	2.00	8.67	10.67	3.47
LSD at 5% level		4.45	0.5138	1.513	1.457	0.44
Sids 12	0	61.33	1.00	5.33	5.30	1.60
	5	74.00	1.33	5.33	6.51	2.31
	10	79.33	2.00	8.67	6.78	2.29
Misr 3	0	84.33	1.33	6.33	10.72	3.77
	5	89.00	1.33	7.00	10.01	3.60
	10	92.00	2.00	8.67	1.50	4.65
LSD at 5% level		6.293	0.727	2.139	2.06	0.625

Table 2: Glycinebetaine effects on photosynthetic pigments (mg/g fresh leaf) of two wheat cultivars under sandy soil conditions

Cultivars	GB (mM)	Chlorophyll A	Chlorophyll B	Carotenoids	Total photosynthetic pigments	A + B	A / B
Sids 12		1.707	0.482	0.385	2.348	2.189	3.544
Misr 3		2.149	0.583	0.443	2.939	2.732	3.694
LSD at 5% level		0.063	0.022	0.012	0.038	0.082	0.089
	0	1.714	0.469	0.347	2.328	2.183	3.649
	5	1.907	0.525	0.406	2.557	2.431	3.626
	10	2.163	0.604	0.491	3.046	2.767	3.581
LSD at 5% level		0.077	0.027	0.014	0.047	0.100	0.109
Sids 12	0	1.569	0.443	0.314	2.151	2.012	3.545
	5	1.697	0.480	0.400	2.344	2.177	3.533
	10	1.856	0.523	0.442	2.551	2.378	3.553
Misr 3	0	1.858	0.495	0.379	2.505	2.353	3.752
	5	2.116	0.596	0.412	2.769	2.686	3.719
	10	2.471	0.685	0.539	3.542	3.156	3.610
LSD at 5% level		0.109	0.038	0.200	0.066	0.142	0.154

Regarding grains yield and its components, both GB treatments significantly increased grains yield and components of two wheat cultivars under investigation (Table3). 5 mM GB increased grains yield of Sids 12 cultivar by 11.33% and Misr 3 by 17.32% whereas, 10 mM GB increased grains yield of Sids 12 cultivar by 21.02% and Misr 3 by 17.88 % relative to corresponding controls. Hence we can say the increase in grains yield of Sids 12 cultivar due to 10 mM GB was higher than 5 mM GB i.e. about twice. On the other hand, increasing in grains yield of Misr 3 cultivar due to both level of GB was approximately similar. It is worthy to mention that adaptation of plants belong to Misr 3 cultivar to conditions of sandy soil was better than that of Sids 12 cultivar. Meanwhile, response of Sids 12 cultivar to 10 mM GB was more effective than response of Misr 3 cultivar.

Table 4 indicates that both GB levels significantly increased most of the investigated biochemical parameters. 10 mM GB showed more pronounced effect than 5 mM GB in both cultivars. The significant increase in total carbohydrate content of Sids 12 cultivar by 5 mM GB was 2.46% and in Misr 3 was 2.91% relative to corresponding controls. Likewise, the significant increase in total carbohydrate content of Sids 12 cultivar by 10 mM GB was 3.83% and in Misr 3 was 6.32% relative to corresponding control.

Table 3: Glycinebetaine effects on grains yield and components of two wheat cultivars under sandy soil conditions

Cultivars	GB (mM)	Spike length (Cm)	Spikes number/ m^2	Spike weight (g)	Grains number / spike	grains weight/spike (g)	100 grains weight	Straw weight (g)	Grains yield (Kg/feddan)
Sids 12		10.47	87.89	2.90	54.33	2.53	4.87	124.90	1280.78
Misr 3		11.55	95.89	4.28	71.78	3.67	5.20	143.59	1387.11
LSD at 5% level		0.556	4.65	0.362	3.477	0.230	0.193	6.367	17.947
	0	9.08	76.00	2.71	51.17	1.98	4.64	116.72	1198.50
	5	11.65	90.50	3.62	65.67	3.19	5.15	125.24	1372.00
	10	12.30	109.17	4.44	72.33	4.13	5.32	160.77	1431.33
LSD at 5% level		0.681	5.70	0.443	4.258	0.282	0.236	7.798	21.981
Sids 12	0	8.40	74.00	1.97	42.00	1.70	4.42	112.55	1156.00
	5	11.33	86.33	2.80	54.67	2.60	4.87	118.89	1287.33
	10	11.67	103.33	3.94	66.33	3.29	5.33	143.25	1399.00
Misr 3	0	9.77	78.00	3.45	60.33	2.25	4.85	120.88	1241.00
	5	11.97	94.67	4.44	76.67	3.79	5.42	131.59	1456.67
	10	12.93	115.00	4.93	78.33	4.97	5.31	178.28	1463.67
LSD at 5% level		0.963	8.059	0.627	6.022	0.398	0.334	11.028	31.086

4. Discussion

4.1. Cultivar variation

These variations in two wheat cultivars (Table 1,2,3,4) may be explained according to Sharaan *et al.* [30] who mentioned that wheat cultivars were significantly different in shoot height, spikes number/plant, spike length, number and weight of grains per spike. Likewise, straw, grains, yields and yield components were significantly different due to cultivar variability as mentioned by Zaki *et al.* [31]. Recently, Dawood *et al.* [1] stated that the growth of plants belong to Misr 3 cultivar was characterized by higher quality and quantity than that of Sids 12 cultivar grown under sandy soil conditions.

Table 4: Glycinebetaine effects on some chemical constituents of yielded grains of two wheat cultivars under sandy soil conditions

Cultivars	GB (mM)	Soluble sugar (%)	Total carbohydrate (%)	Polysaccharides (%)	Proline (mg/g)
Sids 12		3.042	65.47	62.42	1.436
Misr 3		3.736	71.58	67.84	1.571
LSD at 5% level		0.099	0.466	0.472	0.119
	0	2.848	66.78	63.93	1.260
	5	3.540	68.58	65.04	1.382
	10	3.778	70.21	66.43	1.868
LSD at 5% level		0.121	0.571	0.578	0.146
Sids 12	0	2.247	64.12	61.87	1.223
	5	3.407	65.70	62.29	1.360
	10	3.473	66.58	63.11	1.723
Misr 3	0	3.450	69.44	65.99	1.297
	5	3.673	71.46	67.79	1.403
	10	4.083	73.83	69.75	2.013
LSD at 5% level		0.171	0.808	0.818	0.207

4.2. Glycinebetaine application

4.2.1. Vegetative growth parameters

GB treatments increased vegetative growth parameters in both cultivars (Table 1). Mahmood *et al.* [32] studied GB positive roles in enhancing root and shoot fresh biomass of wheat. Since GB application increased internal GB concentration and lead to osmotic adjustment and improved cell growth. GB enhanced morphological aspects of soybean with or without stress as mentioned by Rezaei *et al.* [33]. Moreover, GB enhances morphological features of sweet potato such as shoot fresh dry weight, leaf length, and leaf number [34]. GB increased plant height through increasing stomatal conductance, cell maintenance and increasing cell elongation. Exogenous betaine is thought to aid in the scavenging of free radicals and the protection of antioxidant enzymes [35]. It has been reported that exogenously administered GB promotes a wide range of physiological processes, such as the rate of net CO_2 assimilation [36].

4.2.2. Photosynthetic pigments

Applied GB treatments significantly increased all components of photosynthetic pigments relative to corresponding controls (Table 2). GB may work as an anti-transpirant, allowing the plant to access more water for a longer duration of time and facilitating photosynthesis [37]. Application of GB concentrations enhanced internal precursor choline in leaves and prevented chlorophyll breakdown and inhibit chlorophylase enzyme activity therefore chlorophyll content elevated in leaf tissues [38]. GB is located mostly in chloroplast where it plays a vital role in adjustment and protection of photosynthetic apparatus [39] by minimizing photoinhibition [40], partially preserving the net photosystem-II efficiency [17], protecting Rubisco enzyme and lipids of the photosynthetic apparatus and preserving electron transport across thylakoid membranes [39,41] thus improves the resistance of photosynthetic machinery to stressful conditions [22, 42,43].

4.2.3. Grains yield and its components

Both GB treatments significantly increased grains yield and its components of two wheat cultivars under investigation (Table 3). Compatible solutes serve as a regulating or signaling molecule to stimulate different physiological and biochemical functions as well as plant adaptation mechanism under stress conditions [11]. In the cellular compatibility technique, osmolytes replace water in biochemical processes thereby, keeping normal metabolism throughout stress[44].Osmolytes may also have a role in scavenging reactive oxygen species (ROS), protecting macromolecules (nucleic acids, proteins, and lipids), and acting as a carbon and nitrogen supply reservoir [45] thereby increased crop yield. Furthermore, GB could scavenge free radicals, which was more crucial than their activity as an osmolyte [11]. Estaji *et al.* [46] mentioned that under salt stress conditions, GB can play an important role as a regulator to promote photosynthetic rate and hence production of cucumber plants due to increased total soluble carbohydrate, proline and GB content. Exogenous application of GB also results in higher yields mainly due to improved net photosynthesis [43]. Either foliar-applied on wheat plant or grain priming with GB was found to be beneficial in improving wheat cultivars' growth characteristics and grain yield, as well as the levels of some critical metabolites as reported by Shahbaz *et al.* [41]. Maqsood *et al.* [47] mentioned that seed priming with GB increased the growth parameters, chlorophyll contents, gas exchange parameters, enzymatic antioxidants, endogenous GB, and yield in quinoa plant.

4.3. Chemical composition of the yielded grains

Table 4 indicates that both GB levels significantly increased most of the investigated biochemical parameters. These results may be explained by Sakr et al. [48]; Shemi *et al.* [43]; Dustgeer *et al.* [49] who stated that application of osmoregulators increased proline and soluble sugars. According to Cisse *et al.* [50], GB increased salt stress tolerance by modulating antioxidant substances such as phenolic compounds and osmolytes such as proline and soluble sugar, and promoted growth parameters by improving photosynthetic and antioxidant activities. Mousavi *et al.* [51] stated that increase in sugar content may have a role in osmotic adjustment. Furthermore, soluble carbohydrate accumulations enhance a plant's stress resistance [52]. In addition, accumulations of soluble carbohydrates increase the resistance of plant to stress [52]. Ibrahim [53] observed that GB-treated salt stressed sorghum plants collected more soluble sugars than salinity stressed plants alone. Proline accumulation is one of the most common stress-induced changes in plants, and it plays a role in stress resistance processes [54, 55]. Proline has been connected to cellular osmotic stress reduction, ammonia detoxification, protein and/or membrane stabilization, and enhancing the stability of several cytoplasmic and mitochondrial enzymes [56]. Proline accumulation in wheat cultivars could represent an adaptation to compensate for the energy required for growth and survival, allowing the plant to resist stress [52].These increases may be attributed to reduced proline oxidase, proline catabolizing enzymes as mentioned by Debnath [57]. Furthermore, proline has been proposed as a carbon and nitrogen supply for quick stress recovery, as well as a membrane and macromolecule stabilizer and a free radical scavenger [51].

Conclusion

The growth of wheat plants belong to Misr 3 cultivar under sandy soil conditions was more adaptable than those of Sids 12 cultivar. Both levels of GB have positive effect in reducing the effect of sandy soil conditions and reflected on promotion in vegetative growth parameters, total photosynthetic pigments, grains yield quality and quantity. Moreover, 10 mM GB is considered the optimum treatment for both wheat cultivars.

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