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Physiological role of glycine betaine on sunflower (*Helianthus annuus* L.) plants grown under salinity stress

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Abstract: A pot experiment was carried out at the wire house of the National Research Centre, Dokki, Cairo, Egypt to elucidate the physiological effect of Glycine betaine (0, 2.5, 5 and 7.5 mM) as GB0, GB1, GB2 & GB3 on alleviation the adverse effects of diluted sea water at 3.85 and 7.69 dS/m levels (S1 & S2) on two cultivars of sunflower plants. Low concentration of sea water (3.85 dS/m) increased the studied growth parameters, meanwhile, high concentration (7.69 dS/m) decreased them. Meanwhile, the two levels of diluted sea water decreased photosynthetic pigments, yield, yield components, oil% and protein % of the yielded seeds of sunflower two cultivars compared with plants irrigated with tap water. In contrast, increasing sea water levels led to increases in total phenolics, free amino acids, proline and total soluble sugars contents. Special attention was paid to the effect of GB treatments on salt stressed sunflower that stimulates plant salt tolerance via improving growth parameters, photosynthetic pigments, free amino acids, proline, phenolic and total soluble carbohydrate contents relative to their corresponding salinity controls, thus increasing yield and yield components. From these results, pre-sowing sunflower seeds with glycine betaine seem to enhance sunflower salt tolerance by improvement of photosynthetic pigments, osmoprotectants of vegetative organs, hence improved plant growth and consequently improved yield quantity and quality. Fatty acid profile of sunflower oil show some changes under the effect of salinity and GB treatments.
Key words: fatty acid, glycine betaine, *Helianthus annuus* L., oil, osmoprotectant, proline, salinity stress.

Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crop, it is the forth after soybean, palm oil and canola as a source of edible oil in the world[1]and in the Mediterranean areas where salinity is an increasing problem[2]. Due to increased population of the world, the domestic demand of vegetative oils increased so the promotion of sunflower could be successful to increase the domestic production[3]. Sunflower has gained much attention in order to meet the increasing demand for vegetable oil, especially it could be cultivated in different types of soils and climate conditions as in the newly reclaimed soils and soils irrigated with saline water[4]. Sunflower can tolerate salinity up to EC equal to 1.7 dsm^{-1} so it is moderately sensitive to salinity. Sunflower is high yielding, non conventional oilseed crop. It is a short duration crop (90-120 days) and can be grown twice a year. It fits well in existing cropping systems and can be grown without replacing any major crop[5].

One of the most serious problems in agriculture and the natural status of the environment is salinity. In arable land it is expected to increase salinisation and thus cause devastating global effects, resulting in 30% land loss within the next 25 years, and up to 50% by the year 2050[6]. In the most productive areas of the world, salinity can limit crop yield severely[7]. The effect of salt on plant with time and degree of ions accumulation that rises to toxic level and impose an addition stress on physiological process[8]. High salt content, especially chloride and sodium sulphates, modifying morphological, anatomical[9] and physiological traits including modification of ion balance, water status, mineral nutrition, stomatal behavior, photosynthetic efficiency, and carbon allocation and utilization[10] and this result on plant growth. It is important to prevent cellular damage and to re-establish homeostatic conditions in the new, stressful environment, to improve plant salt tolerance[11]. Plants have developed different adaptive mechanisms to reduce oxidative damage resulting from salinity stress, via biosynthesis of osmoprotectants, such as proline and soluble sugar. Osmoprotectants affect on osmoregulation of cell and protect the structure of different biomolecules and membranes[12] or act as free-radical scavengers that protect DNA from damaging effects of ROS[13]. Proline and soluble sugar accumulation has been reported with salt and drought stresses[14&15]. Phenolic compounds also play an important role in scavenging free radicals and protect plants against the damaging effects of increased ROS levels due to salt and drought stresses[16]. Improving plant tolerance to limited water supplies and high salinity is important to increase their productivity. Recently, great attention has been focused on natural and safety osmoprotectant substances, which have the ability to scavengers ROS forming a protective screen around plant cells, and so improve plant tolerance to stress. Among these natural compounds, glyceinebetaine (GB). GB is a small, highly water soluble molecule that is uniformly neutral, even if present at high concentrations. Glycine betaine has an osmoregulatory role, can stabilizes cell structures and enzyme activities, protects functional proteins, and maintains the integrity of cell membranes against different stressors[17]. GB maintains the turgor pressure during water stress conditions via increasing the equilibrium of water potential maintenance in the cell. In many plants the natural accumulation of GB is lower than sufficient to ameliorate the adverse effects of dehydration caused by various environmental stresses[18]. So exogenous application of GB to low-accumulating or non-accumulating plants can help in reducing the adverse effects of environmental stresses[19]. Externally-applied GB can rapidly penetrate through leaves and transported to other organs, where it might contribute to improve stress tolerance[13]. GB was found to be phloem-mobile, and is partly translocated with assimilates to actively growing shoots and developing organs. Exogenous application of GB has been reported to enhances water stress tolerance in barley[20], sorghum[21], sunflower[22] and canola[14]. The effects of GB on plants vary in response to crop, cultivar, rate and application timing and environmental/location effects. GB was also reported to be highly stable in plant tissues remaining unmetabolized up to 17 days after application[20]. The major role of GB in plants exposed to saline stress is probably protecting plant cells from salt stress by osmotic adjustment[23], protein stabilization[24] (RuBisCo), photosynthetic apparatus protection[25].

Thus, the aim of the present investigation was to assess the efficiency of glycine betaine in alleviating salinity stress on two sunflower cultivars through their actions on growth, photosynthetic pigments, some osmolytes, yield, oil and protein contents of the yielded seeds as well as fatty acids composition.

Materials and Methods

Plant materials and growth conditions

A pot experiment was carried out in two successive seasons April 2014 and 2015 in the wire house of National Research Centre, Dokki, Giza, Egypt. The experimental plant used was sunflower (*Helianthus annuus* L.) cultivars Sakha 53 and Giza 102 were obtained from Agricultural Research Centre, Ministry of Agriculture and Land Reclamation, Egypt. Glycine betaine (GB) used in the present work were supplied from Sigma – Aldrich. Ten uniform seeds were selected by choosing those of equal size and with the same color. Then washed with distilled water, sterilized with 1% sodium hypochlorite solution for about 2 min and thoroughly washed again with distilled water. Sunflower seeds were soaked for 12 h in the different concentrations of glycine bataine 0, 2.5, 5.0 and 7.5 mM considered as GB0, GB1, GB2 or GB3, respectively. Ten uniform, air-dried sunflower seeds were sown in pots (50 cm in diameter) at a depth of 30 mm, in approx. 7.0 kg of clay soil. The soil was mixed with yellow sand in a proportion of 3:1 (v:v) to improve drainage and reduce compaction. Seeds of sunflower were sown in split-split plot design. Cultivars in the main plot, salinity in the subplot and glycine betaine concentration in the sub sub plot, 5 replicates for each treatment were concluded. 20.5% N of granular ammonium sulfate at a rate of 40 kg N ha-1, and single super phosphate (15% P_2O_5) a rate of 60 kg

P_2O_5 ha^{-1}, were added to each pot. The N and P fertilizers were mixed thoroughly into the soil of each pot immediately before sowing. At 15 days after sowing was carried out and 5 plants were left in each pot. Seawater was dissolved in fresh water, to induce salt stress, and the plants were watered with an equal volume of 0.23, 3.85 and 7.69 dS/m, 3 weeks after sowing (treatments S0, S1, and S2, respectively). Saline water was prepared by mixing fresh water (0.23 dS m^{-1}) with seawater (51.2 dS m^{-1}) to achieve salinity levels of 3.85 and 7.69 dS/m. Concentration of EC, pH, cations and anions of irrigation water and soil used were determined[26] and shown in Table 1.The soil water capacity was estimated by saturating the soil in each pot with water and weighing the soil after the soil had drained for 48 h. The water capacity of the soil in each pot was 0.36 kg kg^{-1}. Soil water contents were maintained at approx. 90% of the pot water capacity. The level of soil moisture was controlled by weighing each pot, and any loss of water was supplemented daily.

Table 1. EC, pH, and concentration of cations and anions of irrigation water and soil used in the pot

	EC dSm^{-1}	pH	Cations meq l^{-1}				Anions meq l^{-1}			
			Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	HCO^{-}_3	CO_3^{-}	SO_4^{2-}	Cl^{-}
Soil: Sandy Clay										
	0.16	8.01	2.28	2.67	1.43	0.17	1.10	0.00	4.18	0.74
	1.45	7.73	5.59	1.90	5.81	0.30	1.47	0.00	6.78	5.55
Water: Tap water Sea water										
	0.23	7.36	1.02	0.55	2.42	0.21	0.11	0.00	1.32	2.65
	51.13	7.62	43.25	15.06	454.82	1.53	6.08	0.00	76.38	432.22

Data Recorded

Samples were taken at vegetative growth stage (after 45 days from sowing) to determine shoot height, number of leaves/plant as well as fresh and dry weight of shoot /plant. Photosynthetic pigments and phenolic contents were determined in fresh leaves. Whereas total soluble carbohydrates, free amino acids and proline contents were determined in dry leaves. At harvest, plants were taken to determine head diameter (cm), seeds weight/head (g) and 100-seeds weight (g). Plants were harvested and their heads were air dried and threshed to estimate oil% and protein% of the yielded seeds.

Chemical analysis

Photosynthetic Pigments

Total chlorophyll a, b and carotenoids contents in fresh leaves were estimated using the method[27]. Fresh tissue was ground in a mortar and pestles using 80% acetone. The optical density (OD) of the solution was recorded (for chlorophyll a, b and carotenoids) at 662, 645 nm and 470 nm using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). The values of photosynthetic pigments were expressed in mg/g FW.

Total phenol content

A known weight of the fresh samples was taken and extracted with 85% cold methanol (v/v) for three times at 0°C. The combined extracts were collected and made up to a known volume with cold methanol. 0.5 ml of the extraction was added to 0.5 ml Folin, shacked and allowed to stand for 3 min. Then one ml of saturated sodium carbonate was added to each tube followed by distilled water shaken and allowed to stand for 60min. The optical density was determined at wave length of 725 nm using spectrophotometer[28]..

Total soluble sugars (TSS)

Total soluble sugars (TSS) were extracted by overnight submersion of dry tissue in 10 ml of 80% (v/v) ethanol at 25°C with periodic shaking, and centrifuged at 600g. The supernatant was evaporated till completely dried then dissolved in a known volume of distilled water to be ready for determination of soluble carbohydrates[29]. TSS were analyzed by reacting of 0.1 ml of ethanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 72% H2SO4) in boiling water bath for ten minutes and reading the cooled samples at 625 nm using Spekol SpectrocololourimeterVEB Carl Zeiss.

Free amino acids

Free amino acid content was extracted[30] and determined with the ninhydrin reagent method[31]. One ml acetate buffer (pH 5.4) and 1 ml chromogenic agent were added to 1 ml free amino acid extraction. The mixture was heated in boiling water bath for 15 min. after cooled in tap water, 3 ml ethanol (60% v/v) was added. The absorbance at 570 nm was then monitored using Spekol Spectrocololourimeter VEB Carl Zeiss.

Proline

2.0 ml of proline extract, 2.0 ml of acid ninhydrin and 2.0 ml of glacial acetic acid were added and incubated for 1 h in a boiling water bath followed by an ice bath. The absorbance was measured at 520 nm using Spekol Spectrocololourimeter VEB Carl Zeiss. A standard curve was obtained using a known concentration of authentic proline[32].

Protein contents

Total protein concentration of the supernatant was determined with bovine serum albumin as a standard[33]. An amount of 2 gm of samples were grinded in mortar with 5ml of phosphate buffer (pH 7.6) and was then transformed to the centrifuge tubes. The homogenate was centrifuged at 8000 rpm for 20 minutes. The supernatant of different samples were put in separate tubes. The volume of all of the samples in tubes were then made equal by adding phosphate buffer solution and the extraction were stored in the refrigerator at 40c for further analysis. After extraction, 30µl of different samples were taken out in separate tubes and were mixed with 70µl of distilled water separately. In all of these separate sample tubes 2.9 ml of Coosmassic Brillaint Blue solution was then added and mixed thoroughly. The Total volume now was 3ml in each tube. All these tubes were incubated for 5 minutes at room temperature and absorbance at 600 nm was recorded against the reagent blank. A standard curve of Absorbance (600 nm) versus Concentration (µg) of protein was calculated.

Oil determination

The oil of sunflower seeds were extracted[34], the powdered seeds is shaken overnight with isopropanol : chloroform (1:1). The solvent were evaporated under reduced pressure of CO2 atmosphere. The lipid residue is taken up in a chloroform : methanol (2:1 v/v) and given a folch wash, the dissolved total oils were purified by washing with 1% aqueous saline solution. The aqueous phases were washed with chloroform that was combined with the pure oil solution. Chloroform was evaporated and the total pure oil was weighed.

Fatty acid determination

As the quality of the oil depends on the proportion of different fatty acids, their composition was determined quantitatively by Gas Liquid Chromatography[35].

Statistical analysis of the data

All data were subjected to an analysis of variance (ANOVA) for a factorial design, after testing for the homogeneity of error variances[36]. Statistically significant differences between means were compared at 0.05 using Least Significant Difference (LSD) test.

Results and Discussion

Changes in growth parameters

The obtained results presented in Table (2) show that, low salinity levels increased all growth characters (plant height, leaf number per plant, shoot fresh and dry weights) of the two sunflower cultivars (Sakha 53 and Giza 102). Meanwhile, higher levels decreased these growth parameters compared with control plant. The reduced effect of diluted sea water on plant growth has also been reported on different plant species[37,38,39,40,41&42]. These growth reductions in response to salinity was due the reduced ability of plant cells to absorb water and some mineral nutrients dissolved in the soil[43]. On the other hand, soaking sunflower seeds in glycine betaine with different concentrations caused significant increases in all growth parameters under investigation compared with their corresponding controls (GB0). Data clearly show the superiority of Giza 102

cultivar in all studied parameters over Sakha 53 cultivar. 5.0 mM of glycine betaine was the most effective concentrations of the used concentrations on plants irrigated either with tap water or the two used salinity levels. These increases in plant growth due to soaking seeds of compatible osmolytes mainly glycine beatine may be attributed to the active role of these osmolytes in plant osmotic adjustment, which, in turn, enhanced water uptake and improved growth of plants. In addition the positive role of exogenously applied GB on plant growth of different plant species under stress might be due to its role as a nutrient as well as its role as an osmoprotectant[14].

Table 2. Effect of glycine betaine at 2.5 mM (GB1) and 5.0mM (GB2) and 7.5mM (GB3) on growth parameters of sunflower plants irrigated with tap water (S0) or diluted seawater at 3.85 dS/m (S1) and 7.69 dS/m (S2). The presented results are means of the measurements taken at two successive seasons.

Treatment		Shoot length (cm)		Leaves number/plant		Fresh wt/plant (g)		Dry wt/plant (g)	
Salinity EC(ds/m)	GB (mM)	Sakha 53	Giza 102	Sakha 53	Giza 102	Sakha 53	Giza 102	Sakha 53	Giza 102
S0	GB0	48.35	52.35	12.98	14.39	12.35	14.35	1.34	1.65
	GB1	50.65	54.36	14.96	16.75	14.65	15.98	1.87	1.98
	GB2	53.65	57.68	15.68	17.85	16.35	17.25	2.02	2.14
	GB3	51.35	56.35	15.02	16.54	15.75	16.35	1.89	2.03
S1	GB0	49.36	53.75	13.42	14.96	13.48	14.98	1.45	1.59
	GB1	52.68	55.36	15.35	16.79	15.68	16.02	1.96	2.15
	GB2	54.68	57.98	16.35	18.00	17.85	17.35	2.13	2.35
	GB3	53.68	55.98	15.89	17.35	16.98	17.06	1.96	2.19
S2	GB0	45.68	46.35	10.42	12.36	10.39	11.35	1.13	1.27
	GB1	49.35	51.35	12.68	13.52	12.35	12.74	1.42	1.57
	GB2	52.35	54.68	13.78	14.85	13.75	14.36	1.49	1.63
	GB3	51.98	52.78	12.97	14.01	13.98	14.05	1.35	1.58
LSD @ 5%		2.879		0.641		0.397		0.087	

Changes in photosynthetic pigments

Of many different biochemical attributes, leaf chlorophyll is the most important feature that reflects the health status of plants and that is related to the plant water availability and to the nutrition level. Irrigation of sunflower plants with two diluted sea water (S1 or S2) caused marked gradual decreases in chlorophyll a, chlorophyll b, carotenoids and total photosynthetic pigments in leaves as the salinity level increased compared with those plants irrigated with tap water (S0) (Fig 1). The deleterious effects of salinity stress on leaf chlorophyll contents have been reported in several crops, such as sunflower[44] and flax[45&46]. The reduction in chlorophylls content in response to different stresses might be due to thylakoid membranes disorganisation with more degradation than synthesis of chlorophyll via the formation of proteolytic enzymes, such as chlorophyllase, which is responsible for degrading chlorophyll, as well as damaging the photosynthetic apparatus[47], reducing the plant photosynthetic rate[48]. Application of GB1, GB2 and GB3 caused marked increases in all components of photosynthetic pigments compared with control plants (GB0) irrigated with either tap water or S1 or S2. It was mentioned that exogenous application of GB increased photosynthetic pigments of maize plants[49]. Glycine betaine treatments had the ability to alleviate the adverse effects of salinity on photosynthetic pigments. GB not only functioned as a nutrient but also possessed some defensive mechanisms for damaged plants under salt stress[50], these mechanisms were, promoting photosynthesis, maintaining enzyme activity and scavenging ROS. The beneficial effect of applied GB might be due to the stimulative effects on photosynthetic capacity by overcoming stomata limitations, enhancing biosynthesis of photosynthetic pigments, or protecting photosynthetic pigments from water stress-induced degradation[14].

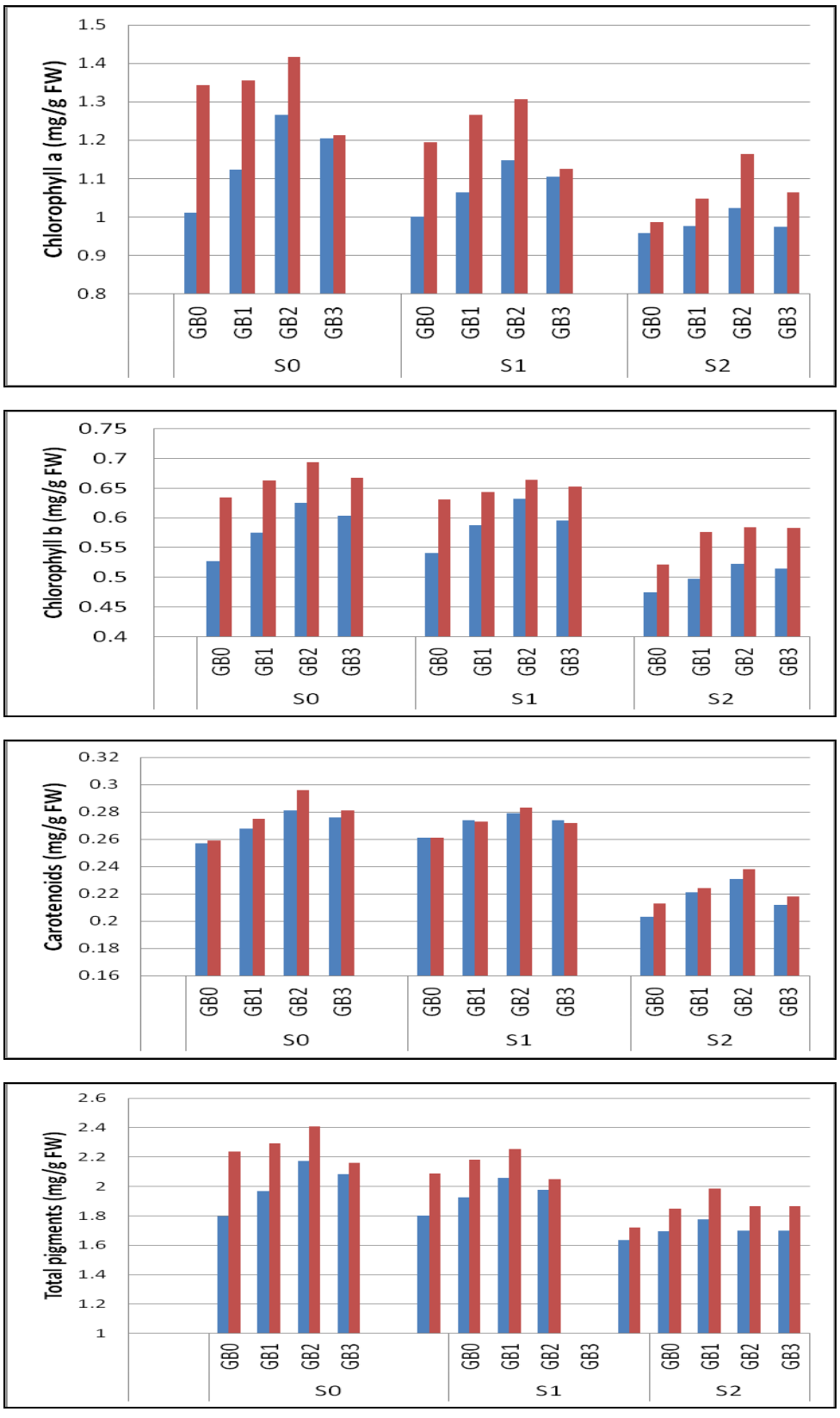

Fig (1): Effect of glycine betaine at 2.5 mM (GB1) and 5.0mM (GB2) and 7.5mM (GB3) on photosynthetic pigments (mg/g FW) of sunflower plants irrigated with tap water (S0) or diluted seawater at 3.85 dS/m (S1) and 7.69 dS/m (S2). The presented results are means of the measurements taken at two successive seasons. LSD at 5% for Chlorophyll a: 0.124, Chlorophyll b: 0.052, Carotenoids: 0.026 and Total pigments: 0.254.

Changes in phenolic contents

Fig 2 shows that the two applied salinity levels (S1GB0 and S2GB0) caused significant and gradual increases in phenolic contents of sunflower leaves (compared with control plants (S0ME0). Glycine betaine concentrations (GB1, GB2 and GB3) caused more gradual increases in phenolic content relative to their corresponding controls. These increases in phenolic compounds synthesis may be the result of some metabolic processes disturbances induced by salt stress[51].

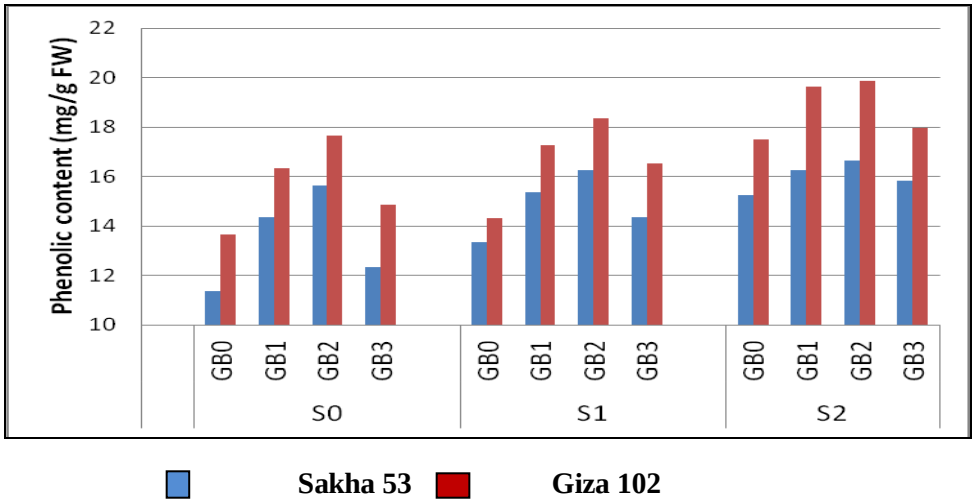

Fig (2): Effect of glycine betaine at 2.5 mM (GB1) and 5.0mM (GB2) and 7.5mM (GB3) on phenolic contents (mg/g FW) of sunflower plants irrigated with tap water (S0) or diluted seawater at 3.85 dS/m (S1) and 7.69 dS/m (S2). The presented results are means of the measurements taken at two successive seasons. LSD at 5%: 1.985.

Changes in free amino acids, proline and total soluble sugars,:

Compatible organic solutes as free amino acids, proline and total soluble sugars accumulate in greater quantity under salinity stress. These solutes stabilize plant membranes, tertiary structures of enzymes and proteins and enhance plant tolerance[37]. Data presented in Fig (3) shows that different salinity levels increased significantly free amino acids, proline and total soluble sugars contents of sunflower plant relative to control plants (S0GB0). These obtained data are in accordance[3,38,15&52]. The low ionization potential that enable proline to form reversible charge- transfer complex with $^1O_2$ and quench this ROS that give proline the protective role[53]. In addition, the reduced proline oxidase (proline catabolizing) enzymes might lead to these increases in proline contents[54]. Moreover, proline has been considered as a carbon and nitrogen source for rapid recovery from stress and acting as stabilizer for membranes and some macromolecules and also as a free radical scavenger[55]. The increased content of TSS in response to salinity stress might be due to the role of TSS on maintaining cell turgidity and overcome the increased resistance to uptake of water in roots. In addition, these increases in TSS under sea water stress may be considered as a protective functions under stress[56]. Moreover, Fig 3 shows clearly that GB treatments significantly increased total soluble sugars, free amino acids and proline concentrations in sunflower leaves under salinity stress as well as in unstressed plants relative to corresponding controls. The accumulation of free amino acids, proline and total soluble sugars under stress and GB treatment is consistent with the early findings on different plant species[57&14].

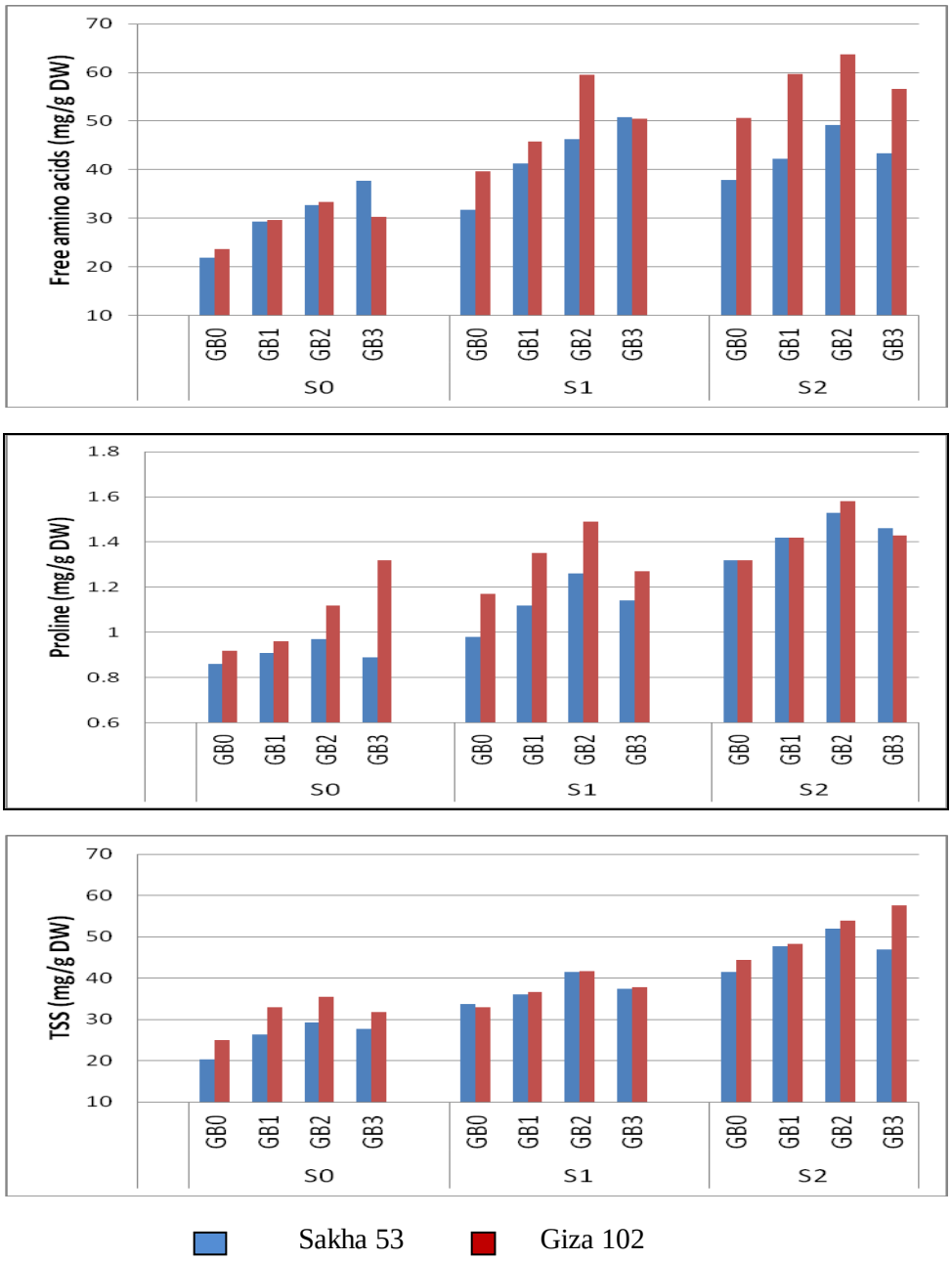

Fig 3. Effect of glycine betaine at 2.5 mM (GB1) and 5.0mM (GB2) and 7.5mM (GB3) on free amino acids, proline and total soluble sugars (TSS) (mg/g DW) of sunflower plants irrigated with tap water (S0) or diluted seawater at 3.85 dS/m (S1) and 7.69 dS/m (S2). The presented results are means of the measurements taken at two successive seasons. LSD at 5% for free amino acids: 1.968, proline: 0.028 and TSS: 1.758. proline:0.028

Changes in yield, yield components, oil% and protein%:

Table 3 show that, salinity stress decreased yield and yield components of the two sunflower cultivars. This effect was more pronounced with cultivar Sakha 53, as the percentages of decreases reached to 11.14%, & 17.72% in head diameter, 13.48% &23.78% in seeds wt /head and 11.80% & 16.55% in 100 seeds wt at S1 &S2 verses, 5.79%& 13.55%, 11.15% & 16.34% and 8.99% & 13.98% of cultivar Giza 102 compared with control plants. The reduction in response to different salinity levels in yield and yield components were concomitant with the decreases in growth parameters (Table 2) and photosynthetic pigments (Fig 1).These decreases in yield might be due to the harmful effect of salinity on growth and the disturbance in mineral uptake[46&42]. In addition, yield is the result of integration reactions in plant, So any change in the metabolic activity at any period of plant growth in response to any influence can affect the yield[14]. Salinity stress

decreased no of heads/plant, head diameter and 100 seed wt of sunflower plant[44]. The used concentrations of GB improved yield and yield components of both tested cultivars under normal & salinity stress conditions (Table 3). GB2 concentration was the most effective concentration at different salinity levels compared with corresponding controls. Cultivar Giza 102 found to be superior in all yield and yield components. These increases in yield and yield components of sunflower plants treated with GB were reported on wheat plant[58] and canola plant[14]. Glycine betaine enhancement effect on growth and yield under normal and stressed conditions due to its osmoprotective role on photosynthesis and ion homeostasis regulation[59]. As well as improving CO_2 assimilation in plant under stress. Also, because of its role in plant growth regulators biosynthesis and transport like cytokinins which have a role in photoassimilates transport[60]. The obtained results in Fig (4) shows clearly that the trend of oil and protein contents of the two sunflower cultivars were correlated to photosynthetic pigments (Fig 1) as well as yield and yield attributes (Table 3). It was noted that, oil and protein contents of the two tested sunflower cultivars were decreased under all different salinity levels. Decreasing oil % and protein% of two sunflower cultivars (Fig 4) with increasing salinity could be mainly attributed to the reductions in seed yield per plant (Table, 3).

Table 3. Effect of glycine betaine at 2.5 mM (GB1) and 5.0mM (GB2) and 7.5mM (GB3) on yield and yield components of sunflower plants irrigated with tap water (S0) or diluted seawater at 3.85 dS/m (S1) and 7.69 dS/m (S2). The presented results are means of the measurements taken at two successive seasons.

Treatment		Head diameter (cm)		Seeds wt/head (g)		100-seed wt (g)	
Salinity EC(ds/m)	GB (mM)	Sakha 53	Giza 102	Sakha 53	Giza 102	Sakha 53	Giza 102
S0	GB0	7.90	8.12	13.58	14.26	5.68	6.01
	GB1	8.69	9.02	15.35	16.75	6.21	6.78
	GB2	10.58	11.24	16.78	18.49	6.87	7.25
	GB3	9.28	9.65	15.75	15.36	6.14	6.89
S1	GB0	7.02	7.65	11.75	12.67	5.01	5.47
	GB1	8.59	9.12	14.52	14.25	5.68	5.68
	GB2	9.55	9.95	14.96	16.74	6.12	6.00
	GB3	9.01	9.24	13.47	15.65	5.42	5.75
S2	GB0	6.50	7.02	10.35	11.93	4.74	5.17
	GB1	7.06	7.69	11.74	12.03	4.96	5.17
	GB2	8.35	8.69	12.68	12.96	5.24	5.48
	GB3	7.56	7.96	11.68	12	5.14	5.28
LSD @ 5%		0.598		0.642		0.165	

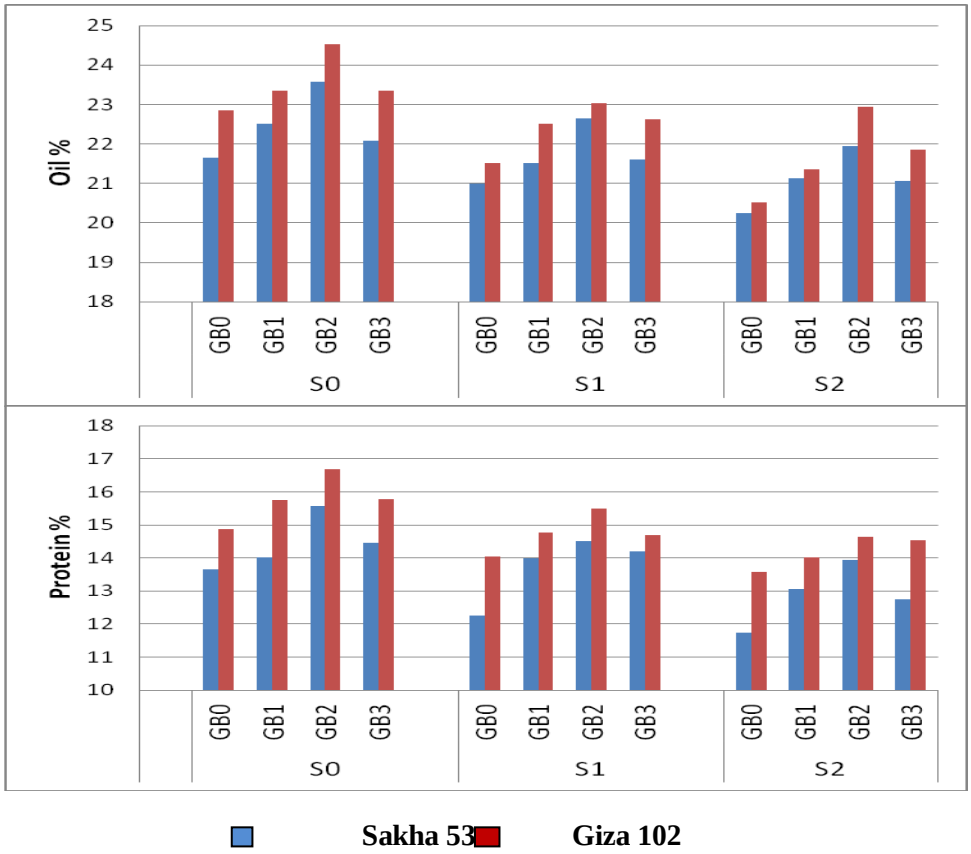

Fig 4. Effect of glycine betaine at 2.5 mM (GB1) and 5.0mM (GB2) and 7.5mM (GB3) on oil% and Protein % of the yielded seeds of sunflower plants irrigated with tap water (S0) or diluted seawater at 3.85 dS/m (S1) and 7.69 dS/m (S2). The presented results are means of the measurements taken at two successive seasons.

LSD at 5% for oil%: 0.345 and for pprotein%: .757

Fatty acid profile of canola oils:

Data in Table (4) show that salinity stress increased saturated fatty acids (palmitic , stearic, arachidic) relative to control plants. Meanwhile, unsaturated fatty acids (oleic, linoleic and linolenic) were decreased by salt stress accompanied by increases in gadoleic acid. Different sea water levels increased total saturated fatty acids and decreased unsaturated fatty acids relative to control plants. Different environmental stresses such salinity have significant effects on seed oil fatty acid composition[61&62]. It is noted that water stress decreased the unsaturation fatty acids which was attributed to the inhibition in the biosynthesis of polyunsaturated fatty acids and suppression in the activities of desaturases[63]. Different responses to GB treatments in unstressed and salt stressed of fatty acid profile of sunflower oils (Table 4). Glycine betaine at 5.0 mM concentration decreased palmitic and stearic acid accompanied with increases in arachidic acid. Meanwhile oleic linoleic and linolenic acids under the interaction effect of GB treatments and normal and salt stress and these results led to decreases in total saturated fatty acid and increases in unsaturated fatty acid relative to corresponding controls. Compatible solutes (GB or proline) improved the oil quantity and quality due to their protective effect on cellular structures during fatty oil biosynthesis and storage, which occurs in liposomes or oleosomes in seeds during seed filling stage[60&62]. It was stated that exogenous GB improved the quality of oil by decreasing the un-saponifiable matter and increasing oil saponification and iodine values, the measure of oil unsaturation[64].

Table 4. Effect of glycine betaine at 2.5 mM (GB1) and 5.0mM (GB2) and 7.5mM (GB3) on growth parameters of sunflower plants irrigated with tap water (S0) or diluted seawater at 3.85 dS/m (S1) and 7.69 dS/m (S2). The presented results are means of the measurements taken at two successive seasons.

Fatty acid	S0GB0		S0GB2		S1GB0		S1GB2		S2GB0		S2GB2	
	Sakha 53	Giza 102	Sakha 53	Giza 102	Sakha 53	Giza 102	Sakha 53	Giza 102	Sakha 53	Giza 102	Sakha 53	Giza 102
16:00	9.7	8.14	9.89	7.47	10.02	9.14	8.53	7.18	10.86	9.24	8.01	7.25
18:00	7.86	7.35	6.74	6.58	8.12	7.95	7.07	6.45	8.69	8.24	6.38	6.34
20:00	1.42	1.74	0.87	0.74	1.67	1.96	1.06	1.27	1.96	2.01	0.00	1.02
16:01	0.75	0.47	0.62	0.47	0.69	0.41	0.95	0.96	0.58	0.35	1.25	1.05
18:01	40.35	53.21	41.35	54.25	38.62	52.45	43.56	54.02	37.32	53.25	44.12	55.01
18:02	35.48	24.68	35.65	26.33	32.52	23.85	37.52	26.52	32.01	24.52	38.12	27.02
18:03	0.58	0.57	0.78	0.98	0.52	0.53	0.78	0.64	0.54	0.58	0.89	0.71
20:01	0.23	0.09	0.34	0.37	0.35	0.12	0.43	0.26	0.24	0.12	0.52	0.32
Ts	18.98	17.23	17.5	14.79	19.81	19.05	16.66	14.9	21.51	19.49	14.39	14.61
Tu	77.39	79.02	78.74	82.4	72.7	77.36	83.24	82.4	70.69	78.82	84.9	84.11
Total	96.37	96.25	96.24	97.19	92.51	96.41	99.9	97.3	92.2	98.31	99.29	98.72
Tu/Ts	4.08	4.59	4.50	5.57	3.67	4.06	5.00	5.53	3.29	4.04	5.90	5.76

Ts) Total saturated fatty acids Tu) Total unsaturated fatty acids
16:0(palmitic);18:0(stearic);18:1(oleic);18:2(linoleic);18:3(linolenic);20:0(arachidic);20:1(gadoleic)

It could be concluded that seed soaking sunflower seeds with glycinebetaine could play an mitigating role and alleviate the adverse effect of salinity stress on many metabolic and physiological processes of two sunflower cultivars that reflected in increasing seed yield quality and quantity.

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