International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.7, pp 78-92, 2016

Physiological role of cyanobacteria and glycinebetaine on wheat plant grown under salinity stress

Mervat Sh. Sadak[1] and Marwa MR. M. Ahmed[2]

[1]Botany Dept, National Research Centre, 33 El-Tahrir St., Dokki, 12622 Giza, Egypt
[2]Botany Department, Faculty of Science, Assiut University, New Valley Branch, Egypt

Abstract : Salinity is an important abiotic stress that reduces growth and productivity of different crops. In many agricultural soils cyanobacteria or blue green algae are the prominent inhabitants, where they potentially contribute to improve soil fertility and crop productivity under normal and abiotic stress conditions. Glycinebetaine is an osmoprtectant compound improving plant tolerance to abiotic stress. Thus, it is very important to study the physiological role of glycinebetaine in mitigating the harmful effects of salinity stress in presence or absence of cyanobacteria under recommended or half recommended doses of NPK fertilizers experienced by wheat cultivar Giza 168. Herein, it was observed that, salinity stress decreased morphological parameters (shoot length, flag leaf area/plant, tillers fresh and dry weight), photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments), yield & yield attributes (plant height, spike number/plant, spike length, spikes weight/plant, grain weight/plant and 1000 grain weight) as well as some biochemical aspects of the yielded grains (carbohydrate%, protein%, nitrogen%, phosphorus% and potassium%). Meanwhile, it increased some osmoprotectant compounds as total soluble sugars, free amino acids and proline contents. Regarding to cyanobacteria and glycinebetaine effect under recommended or half recommended doses of NPK, presoaking of wheat grains with gylycinebetaine 5mM in absence and presence of cyanobacteria and recommended dose and half recommended dose of NPK improved growth and yield attributes of wheat plant grown under salinity stress via increasing plant tolerance to salinity stress by increasing some metabolic activities as photosynthetic pigments, total soluble sugars, free amino acids and proline contents.
Keywords : Cyanobacteria, Glycinebetaine, Osmoprotectants, Salinity, Wheat, Yield.

Introduction

Wheat (*Triticum aestivum* L.) is one of the main food crops and cultivated worldwide firstly as food commodity and secondly a strategic commodity. Due to higher protein content of wheat grain over maize (corn) or rice so wheat is considered the main leading source of vegetable protein in human diet[1].

Salinity is one of the most serious environmental problems that affect adversely on plant growth and productivity of different crops. According to FAO, about one-third of world cultivated areas is affected by salinity stress[2]. Salinity problem is accentuated by competition for high quality of water in agricultural lands and industry, so, landscape users has prompted to use of alternative sources of water of irrigation as diluted sea water. Plants have evolved various mechanisms to survive with the serious effects of salt stress. One of these mechanisms, osmotic adjustment[3,4]. Osmotic adjustment is very important to maintain uptake of water from saline environment. Osmotic adjustment is known as the decrease in osmotic potential of plant cell as a result of

increasing intracellular solutes not from a cell water loss. These might be via increases in inorganic and/or organic solutes[5]. These organic solutes as some neutral compounds and soluble ones that named as compatible solutes as soluble sugars, amino acids, organic acids and inorganic ions[6&7]. Different strategies are used to improve plant tolerance to limited supplies of water and high salt stress and increase plant productivity. One of the used strategies is using of safety and natural osmoprotectant compounds, that have the ability to scavenge the formed reactive oxygen species to improve tolerance of plant. Among of these osmoprotectants glycinebetaine (GB), proline and trehalose[8,9]. Glycinebetaine (N,N,N-trimethyl glycine) is highly soluble, small compound can stabilizes cell structures and activities of different enzymes also, GB play an important role in enhancing plant tolerance under abiotic stress including salinity, drought, heat[10]. Natural GB accumulation in plants is lower than sufficient to alleviate the adverse effects of dehydration caused by different environmental stresses. So exogenous treatment of GB to non accumulating or low accumulated plants may help in reducing the adverse effects of environmental stresses.

Cyanobacteria or blue-green algae, are a diverse group of prokaryotes have an oxygen evolving photosynthetic systems[11]. It has the ability to associate with non-vascular/vascular plants and produce an array of biologically active metabolites[12]. So enhance plant growth under different environmental stresses. The role of cyanobacteria in improving plant growth under different stresses is as an osmoprotective compound and maintaining low internal contents of inorganic ions. These substances are able to protect macromolecules against denaturation[13]. Of many agricultural soils, cyanobacteria are prominent inhabitants and effectively contribute towards biological nitrogen fixation, help in phosphate solubilization and mineral release to improve soil fertility and crop productivity[14]**.** However, beside naturally fertilizing and balancing mineral nutrition in the soil, many cyanobacteria are known to release various kinds of biologically active substances like proteins, vitamins, carbohydrates, amino acids, polysaccharides and phytohormones, which function as signalling molecules to promote plant growth[15]**.** Using of N_2 –fixing cyanobacteria also, is the ultimate goal to decrease the dependence of chemical nitrogen fertilizers for crop production. For a longer time periods, due to indiscriminate use of chemical fertilizers thus, drastically disturb the natural ecological balance[16].

So, the aim of the present work was to impact the physiological role of grain soaking with glycine betaine in absence and presence of cyanobacteria under recommended or half recommended doses of NPK on growth, some biochemical aspects and yield quantity and quality of wheat plant grown under salinity stress. Reducing the amount of chemical fertilizer up to half in the presence of bio fertilizer (cyanobacteria) help to increase the defense system of wheat for all the above mentioned parameters to alleviate the adverse effect of salinity.

Materials and Methods

A pot experiment was conducted at the green house of National Research Centre, Cairo, Egypt, during two successive winter seasons of 2014/2015 and 2015/2016. Grains of wheat plant (cv Giza 168) were obtained from Agricultural Research Centre, Cairo, Egypt. Cyanobacteria were obtained from the Microbiology Department, Soils, Water and Environment Res. Inst., Agric. Res., Center. Glycinebetaine, (GB) were supplied from SIGMA–ALDRICH Company. The aim of this work was to investigate the effect of cyanobacterial addition to the soil (5 g/Kg soil) and/or soaking treatment with glycinebetaine (GB) at 5 mM concentration for 12 hours of wheat grains (Giza 168) cultivar grown under two environmental salinity soil conditions with recommended (R) or half recommended (½R) dose of NPK fertilizer. Ten uniform grains 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. The experimental design was split-split plot design with five replicates. Which salinity levels occupy the main plots, while nitrogen fertilizer treatments were in sub plots and the treatments of (cyanobacteria and glycinebetaine (GB)) were allocated at sub-sub plots. Grains of wheat were sown on the 15 November in both seasons in (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. Fertilization was done with the recommended dose (5g phosphorus/pot as triple phosphate, 6g nitrogen/pot as urea and 5g potassium/pot as potassium sulphate) or half recommended doses (2.5g phosphorus/pot as triple phosphate, 3g nitrogen/pot as urea and 2.5g potassium/pot as potassium sulphate) during preparation of pots and after sowing. At 15 days after sowing thinning was carried out and 5 uniform plants were left in each pot. Saline water was prepared by mixing fresh water (0.23 dS/m) with seawater (51.2 dS/m) to achieve salinity levels of 0.23 and 7.69 dS/m.

Concentration of EC, pH, cations and anions of irrigation water and soil used were determined[17]and shown in Table 1.

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^{-}
Water: Tap water Sea water										
	0.23	7.35	1.00	0.50	2.40	0.20	0.10	0.00	1.30	2.70
	51.2	7.76	43.20	15.12	454.57	1.51	6.05	0.00	76.36	432.00
Soil: Sandy Clay										
	0.14	8.11	2.60	2.40	1.31	0.21	1.13	0.00	4.22	0.70
	1.40	7.59	5.60	1.90	5.90	0.37	1.50	0.00	6.77	5.50

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. 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. Plant samples were taken after 75 days from sowing for measuring growth characters (plant height (cm), flag leaf area (cm^2), tillers fresh and dry weight (g)) and some chemical analysis (photosynthetic pigments, total soluble sugars, free amino acids and proline). At harvest, plants were taken to determine yield and yield components (plant height (cm), spike number/plant, spike length (cm), spikes weight/plant (g), grain weight/plant (g) and 1000 grain weight(g)). As well as some nutritional values of the yielded grains (carbohydrates%, protein%, nitrogen%, phosphorus% and potassium%).

Chemical analysis:

Photosynthetic Pigments

Total chlorophyll a, b and carotenoids contents in fresh leaves were estimated using the method[18]. 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 soluble sugars

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 according to the method described[19]. 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 Spectrocololourimeter VEB Carl Zeiss[20].

Free amino acids

Free amino acid content was extracted according to the method[21]. Free amino acid was determined with the ninhydrin reagent method[22]. 1.0 ml acetate buffer (pH 5.4) and 1.0 ml chromogenic agent were added to 1.0ml 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

Proline was assayed according to the method[23]. Two 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.

Total carbohydrate:

Determination of total carbohydrates was carried out according[24]. A known mass (0.2-0.5 g) of dried tissue was placed in a test tube, and then 10 ml of sulphuric acid (1N) was added. The tube was sealed and placed overnight in an oven at 100ºC. The solution was then filtered into a measuring flask (100ml) and completed to the mark with distilled water. The total sugars were determined colorimeterically according[25]as follows: An aliquot of 1ml of sugar solution was transferred into test tube and treated with 1ml of 5% aqueous phenol solution followed by 5.0 ml of concentrated sulphuric acid. The tubes were thoroughly shaken for ten minutes then placed in a water bath at 23-30ºC for 20 minutes. The optical density of the developed color was measured at 490 nm using Shimadzu spectrophotometer model UV 1201.

Protein contents

Total protein concentration of the supernatant was determined with bovine serum albumin as a standard[26]. 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.

Mineral Ions:

Macroelement contents of the yielded grains were determined Total N was determined by using micro-Kjeldahl method[27]. Phosphorus and potassium were determined[17]. Phosphorus was determined using a Spekol spectrocolorimeter (VEB Carl Zeiss; Jena, Germany, while, estimation of K+ contents were done using a flame photometer.

Statistical analysis:

The data were statistically analyzed using software[28]. The mean comparisons among treatments were determined by Duncan's multiple range tests at 5% level of probability[29].

Results

Morphological parameters:

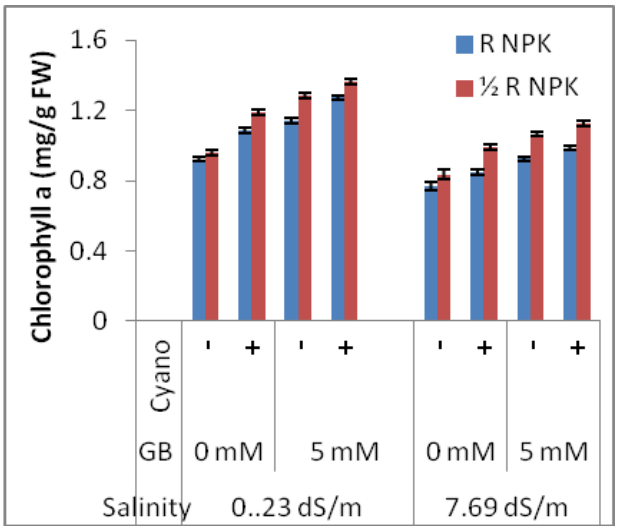
Table (2) show the effect of glycinebetaine (GB) on wheat plant (Giza 168) cultivated in soil amended with cyanobacteria and recommended or half recommended doses of NPK on morphological parameters under sea water stress. Irrigation of wheat plant with diluted sea water (7.69 dS/m) caused significant decreases in morphological parameters (plant height, flag leaf area, tillers fresh and dry weight) in recommended (R) or half recommended dose (½R) of NPK compared with control plant. Meanwhile, cyanobacteria amended to soil increased significantly these morphological parameters of wheat plant compared with those plants cultivated in absence of cyanobacteria. Also, data clearly show that, cyanobacteria in soil with half recommended dose of NPK significantly increased growth parameters of wheat plant compared with those of recommended dose of NPK without cyanobacteria. Regarding to GB effect, soaking wheat grains in glycinebetaine (5mM GB) induced more increases in the studied growth parameters compared with the corresponding salinity in absence and presence of cyanobacteria, recommended dose or half recommended dose of NPK.

Table (2): Effect of cyanobacteria, glycinebetaine (GB) and NPK doses on morphological parameters of wheat plant grown under diluted sea water (Data are means of two seasons)

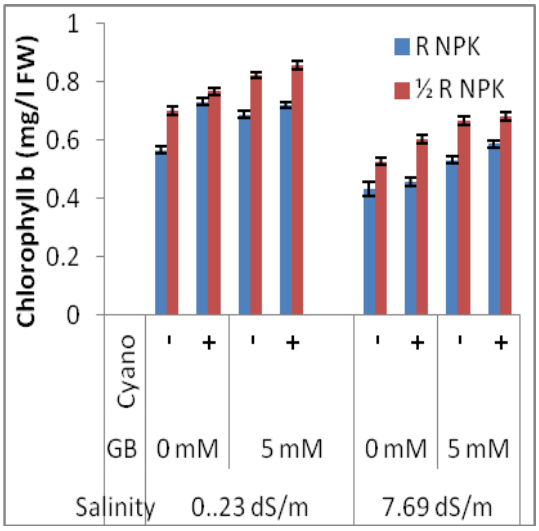
Salinity (dS/m)	GB (mM)	Cyano	Plant height		Flag leaf area (cm^2)		Tiller fresh wt (g)		Tiller dry wt (g)	
		NPK	R	½R	R	½R	R	½R	R	½R
0..00	0.0	-	52.2±1.523	55.5±1.493	30.2±1.244	33.2±1.287	8.3±0.534	9.3±0.144	2.4±0.067	2.5±0.035
		+	56.5±1.545	63.2±1.574	33.6±1.852	39.2±1.357	9.5±0.257	9.8±0.244	2.6±0.093	2.7±0.047
	5	-	62.3±1.814	68.5±1.897	35.5±1.872	42.6±1.323	9.8±0.145	10.5±0.322	2.7±0.084	2.9±0.042
		+	67.2±1.524	72.0±1.787	39.3±1.254	46.5±1.415	10.5±0.243	11.4±0.324	2.8±0.071	3.1±0.044
7.69	0.0	-	31.2±1.022	36.1±1.322	23.5±1.473	27.6±1.244	5.82±0.137	6.1±0.242	1.6±0.058	1.7±0.032
		+	38.5±1.153	43.2±1.474	26.5±1.354	31.2±1.426	6.68±0.542	7.7±0.144	1.7±0.042	1.8±0.034
	5	-	42.3±1.454	46.0±1.423	29.3±1.052	34.2±1.424	7.69±0.327	8.68±0.417	1.8±0.044	1.8±0.032
		+	46.5±1.349	51.5±1.542	32.5±1.063	35.8±1.355	8.14±0.142	9.14±0.412	1.9±0.242	1.8±0.034
LSD at 5%			3.324		2.354		0.452		0.158	

Changes in photosynthetic pigments:

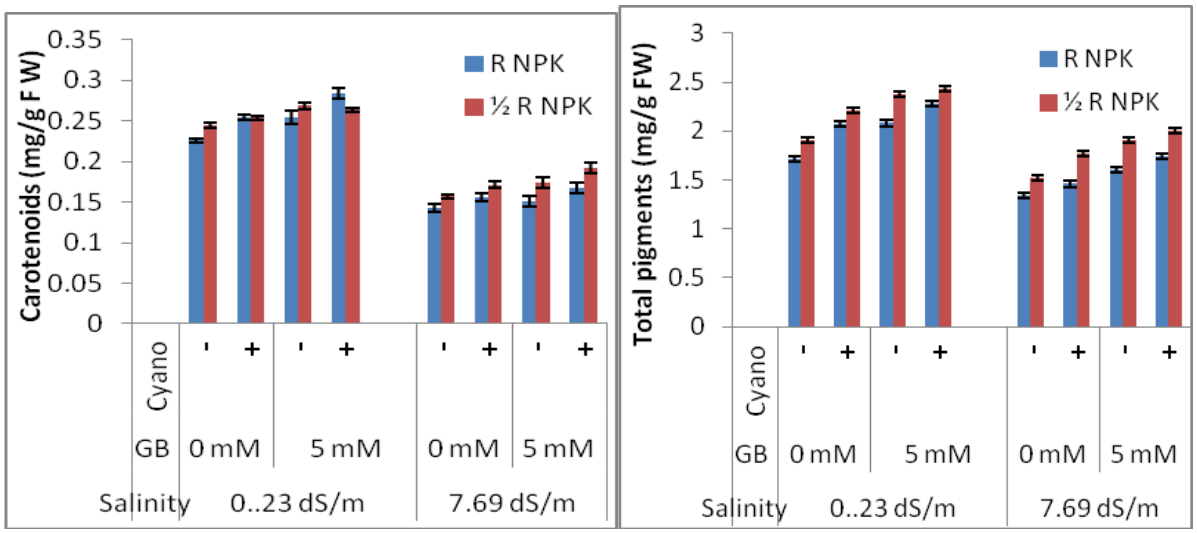
Data presented in Fig (1) shows that, irrigation of wheat plant with diluted sea water (7.69 EC dS/m) caused significant decreases in photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) compared with control plants irrigated with normal water in absence and presence of cyanobacteria, recommended or half recommended doses of NPK fertilizers. Whereas, cyanobacteria amended to soil increased significantly photosynthetic pigments compared with those cultivated in absence of cyanobacteria. As well as, glycinebetaine (GB) treatment not only, enhanced the photosynthetic pigments of wheat plants irrigated with normal water but also, alleviated the reduced effect of salinity stress on photosynthetic pigments compared with untreated plants in absence and presence of cyanobacteria. Maximum increases in photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) were obtained by using triple treatments (cyanobacteria + GB + 100% NPK) over all other treatments. Data clearly show that, cyanobacteria with GB + 1/2 R dose of NPK treatment caused more pronounced increase in photosynthetic pigments of wheat plant as compared with the corresponding control plants with R doses of NPK.



LSD at 5%: 0.042



LSD at 5%:0.027



LSD at 5%: 0.014 **LSD at 5%:0.075**

Fig (1): Effect of cyanobacteria, glycinebetaine (GB) and NPK dose on photosynthetic pigments (Chlorophyll a, chlorophyll b, carotenoids and total pigments) (mg/g FW) of wheat plant under salinity levels (Data are means of two seasons)

Total soluble sugars:

Glycinebetaine effects on wheat plant (Giza 168) cultivated in soil amended with cyanobacteria, recommended or half recommended doses of NPK on the total soluble sugar are shown in Fig 2. Irrigation of wheat plants with diluted sea water (7.69 dSm^{-1}) significantly increased total soluble sugars in wheat plants in recommended and half recommended dose of NPK. Moreover, cultivation of wheat plant in the presence of cyanobacteria led to marked increases in soluble sugars as compared with plants cultivated without cyanobacteria. Data obtained revealed also, that soaking wheat plant with 5mM of glycinebeataine stimulated more accumulation of total soluble sugar as compared with the corresponding salinity levels in the presence or absence of cyanobacteria.

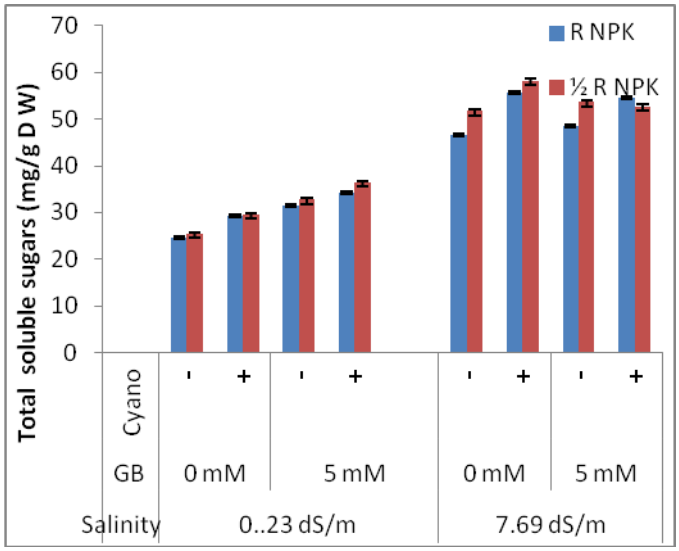


Fig (2): Effect of cyanobacteria, glycinebetaine (GB) and NPK dose on total soluble sugars (mg/g DW) of wheat plant under salinity levels (Data are means of two seasons)

Amino acids and Proline

Data presented in Fig (3): show that salinity stress (9.39 EC dS/m^{-1}) caused significant increases in free amino acids and proline content. Furthermore, GB significantly enhanced the stimulatory role of salt stress on the production of free amino acids and proline in wheat plant in the presence and absence of cyanobacteria. The maximum increases in both free amino acides and proline were obtained by using the triple treatment (cyanobacteria + GB + R dose of NPK) over the all other treatments.

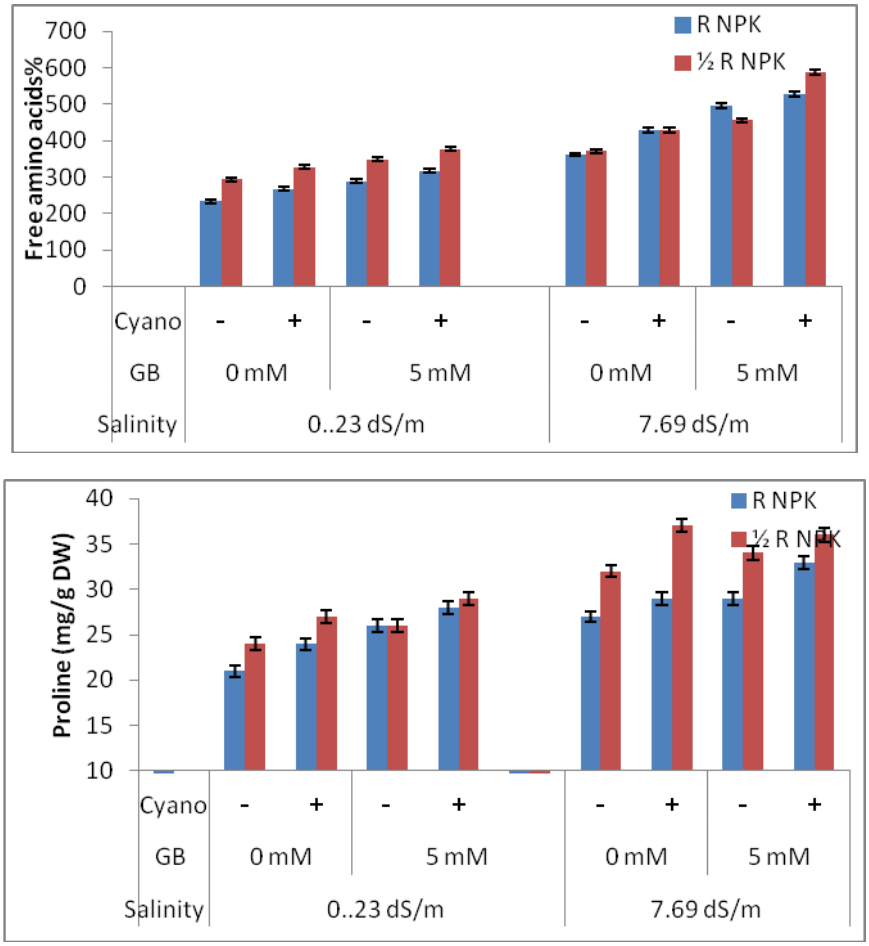


Fig (3): Effect of cyanobacteria, glycinebetaine (GB) and NPK dose on free amino acids and proline (mg/g DW) of wheat plant under salinity levels (Data are means of two seasons)

Yield and yield attributes:

Table (3&4) show the effect of glycinebetaine (GB) on wheat plant (Giza 168) cultivated in absence and presence of cyanobacteria and recommended or half recommended doses of NPK on yield and yield attributes of wheat plant grown under sea water stress. Irrigation of wheat plant with diluted sea water (7.69 dS/m) caused significant decreases in yield attributes (plant height, spike number/plant, spike length, spikes weight/plant, grain weight/plant and 1000 grains weight) compared with plant irrigated with normal water. In the same time, cyanobacteria amended to soil partially or completely alleviated the reduced effect of diluted sea water on yield attributes of wheat plants by increasing significantly these studied parameters compared with those cultivated without cyanobacteria. Data clearly show that, cyanobacteria in soil with half recommended dose of NPK significantly increased yield attributes of wheat plant compared with those of R dose without cyanobacteri. Soaking wheat grains in glycinebetaine (5mM GB) induced more increases in the studied growth parameters compared with the corresponding salinity in absence and presence of cyanobacteria.

Table (3): Effect of cyanobacteria, glycinebetaine (GB) and NPK dose on yield attributes (plant height cm, sike number/plant, spike length cm) of wheat plant under salinity levels (Data are means of two seasons)

Salinity (dS/m)	GB (mM)	Cyano	Plant height (cm)		Spike No/plant		Spike length (cm)	
			R NPK	½R NPK	R NPK	½R NPK	R NPK	½R NPK
0..00	0	-	66.77±0.734	74.88±0.856	3.85±0.021	4.55±0.036	10.08±0.121	12.17±0.214
		+	71.04±0.786	80.61±0.825	4.22±0.045	4.98±0.045	11.22±0.124	13.08±0.201
	5	-	76.84±0.905	91.88±0.942	4.26±0.035	5.03±0.054	11.86±0.214	14.22±0.157
		+	81.77±0.507	85.37±0.937	4.79±0.023	5.47±0.035	13.12±0.421	15.51±0.175
7.69	0	-	45.77±0.584	51.5±0.567	2.86±0.041	3.24±0.047	7.84±0.321	9.23±0.175
		+	52.77±0.584	58.61±0.644	3.25±0.042	3.86±0.042	8.85±0.095	10.42±0.174
	5	-	56.84±0.629	61.38±0.674	3.78±0.042	4.11±0.024	9.78±0.085	11.42±0.185
		+	61.04±0.676	66.61±0.732	4.01±0.025	4.37±0.024	10.75±0.142	11.95±0.174
LSD at 5%			3.324		0.125		1.024	

Table (4): Effect of cyanobacteria, glycinebetaine (GB) and NPK dose on yield attributes (spikes wt/plant g, grain wt/plant g, and 1000 grain wt) of wheat plant under salinity levels (Data are means of two seasons)

Salinity (dS/m)	GB (mM)	Cyano	Spikes wt/plant (g)		grain wt/plant (g)		1000 grains wt (g)	
			R NPK	½R NPK	R NPK	½R NPK	R NPK	½R NPK
0..00	0	-	6.97±0.085	7.26±0.085	3.66±0.042	4.96±0.052	42.70±1.025	45.71±1.047
		+	7.25±0.074	7.66±0.096	4.95±0.042	5.17±0.045	42.57±1.052	48.34±1.052
	5	-	7.83±0.065	7.96±0.085	4.26±0.035	5.4±0.052	46.05±1.024	49.31±1.025
		+	8.17±0.074	8.66±0.96	5.39±0.075	6.56±0.035	48.76±1.025	56.79±1.014
7.69	0	-	4.52±0.068	4.86±0.075	2.72±0.025	3.17±0.054	23.22±0.852	23.41±0.868
		+	5.33±0.075	5.56±0.85	3.13±0.041	3.28±0.035	28.38±0.752	31.58±0.968
	5	-	6.34±0.054	6.96±0.086	3.39±0.025	3.28±0.024	38.06±0.862	50.97±0.862
		+	6.79±0.074	7.66±0.098	3.78±0.025	3.94±0.034	38.83±0.758	51.52±0.952
LSD at 5%			0.558		0.452		0.321	

Carbohydrate and protein percentages in the yielded grains:

Data presented in Fig (4) revealed that, irrigation of wheat plant with diluted sea water (7.69 ds/m) decreased significantly carbohydrate and protein percentages of the yielded grains in amended soil with cyanobacteria and recommended and half recommended doses of NPK as compared with those irrigated with normal water. On the other hand, cyanobacteria amended to soil increased significantly carbohydrate and protein percentages compared with plants grown in absence of cyanobacteria in recommended and half recommended doses. Fig (4) also show that grain soaking of wheat plants in 5mM GB could alleviated the reducing effect of salinity stress by increasing significantly both of carbohydrate and protein percentages.

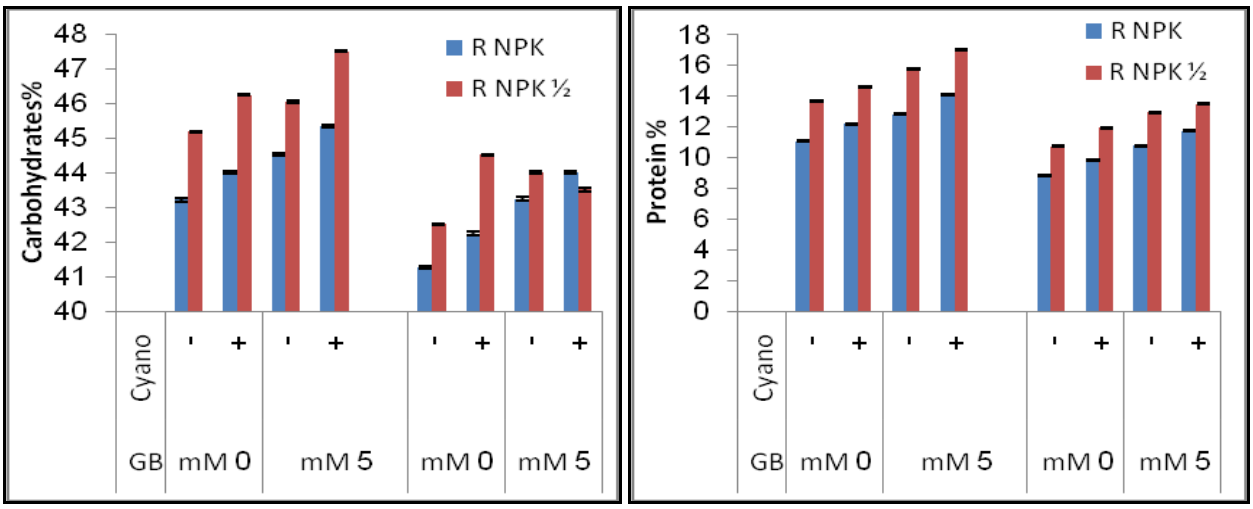
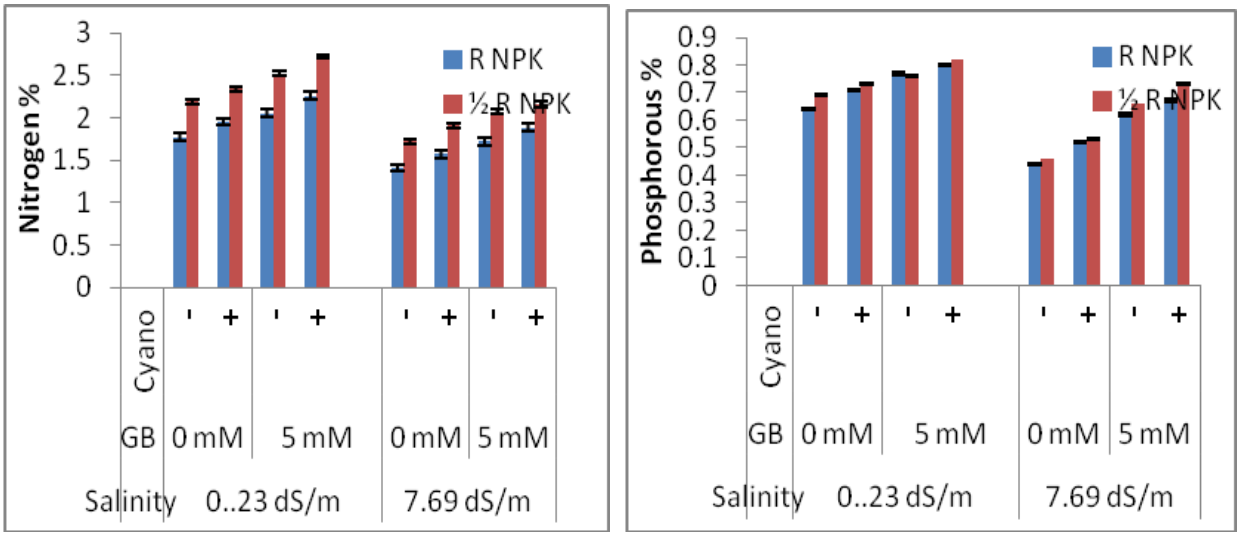


Fig (4): Effect of cyanobacteria, glycine betaine (GB) and NPK dose on carbohydrate% and protein % of the yielded grains of wheat plant under salinity levels (Data are means of two seasons)

Macronutrient contents in yielded grains

With regard to the effect of cyanobacteria, GB on wheat plants grown under salinity stress and recommended and half recommended doses of NPK, Fig (5) show that, diluted sea water decreased significantly nitrogen, phosphorous and potassium percentages in the yielded grains either in presence or absence of cyanobacteria also, in recommended or half recommended doses of NPK as compared with control plants. Meanwhile cyanobacteria amended to soil caused significant increases in N, P and K percentages of the yielded grains as compared with those in absence of cyanobacteria either under recommended or half recommended NPK fertilization. In addition, soaking wheat grains in 5mM GB could partially or completely alleviate the harmful effect of diluted sea water on N, P and K contents as it increased significantly N, P and K percentages of the yielded grains as compared to untreated plants under different salinity levels, in absence and presence of cyanobacteria, recommended or half recommended dose of NPK fertilization level.



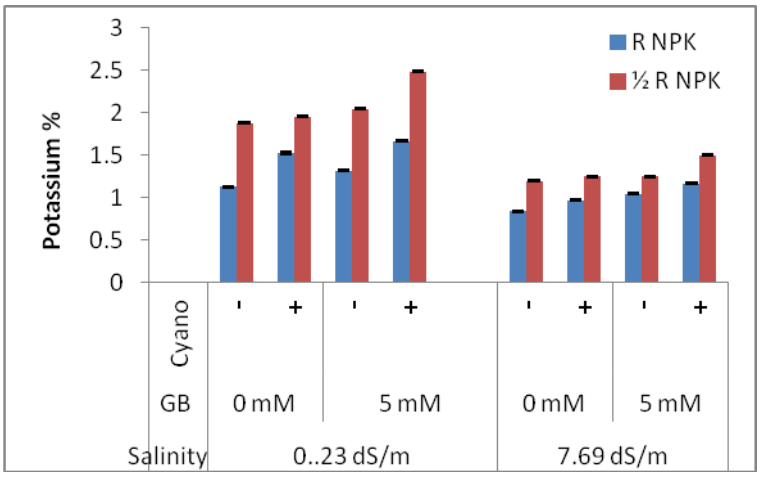


Fig (5): Effect of cyanobacteria, glycinebetaine (GB) and NPK dose on nitrogen, phosphorus and Potassium percentages of the yielded grains of wheat plant under salinity levels (Data are means of two seasons)

Discussion

Salinity stress is one of most abiotic stress that reduce growth of different plants and their productivity. Plant cell is confronted by ionic toxicity, osmotic stress and nutritional disorders[30]. To overcome the degree of cellular damage caused by abiotic stress specially salinity stress and to improve crop tolerance, Different methods are used. Among these, amending soil with cyanobacteria and using of osmoprotecting compounds as glycinebataine (GB) as an external treatment to plant. Our obtained data in Table (2) demonstrates that diluted sea water (7.69 dS/m EC) decreased significantly the studied morphological parameters of wheat plant Giza 168 cultivar compared with control plant that irrigated with tap water. These results are in good agreement with those on faba bean[3], sunflower[8], *Calendula officinal*[31] and wheat plant[7]. Reduced growth parameters of wheat plant in response to irrigation with diluted sea water is due to plant cells reduced ability to absorb water and dissolved nutrients in the soil[32]. On the other hand, cultivation of wheat plant in soils amended with cyanobacteria caused significant increases in the above mentioned morphological criteria compared with those cultivated in absence of cyanobacteria. These results are in agreements with those obtained[33&34]. These increases might be due to that some species of cyanobacteria have the ability on nitrogen fixation as well as cyanobacteria as a new bio fertilizer (containing N, P, K, Ca, Mg, and S as well as Zn, Fe, Mn, Cu, Mo, Co and some growth regulators) applied to improve vegetative growth and yield[35] thus the amount of chemical N fertilizer as well as phosphorus and potassium can be decreased. Moreover, soaking wheat grains in 5mM of GB resulted in more increases in the studied morphological parameters in presence and absence of cyanobacteria as compared with untreated plants. These results are agreed with those obtained on canola plant[36] and sunflower plant[37]. The promotive effect of glycinebetaine as an osmoprotectant compound could be due to the active enhancing role of osmolytes in osmotic adjustment of plant, thus enhancing the uptake of water by plant cells thus improved growth. Moreover, exogenously applied GB enhancing role on different plant species under stress might be due its role as a nutrient as well as its role as an osmoprotectant[38].

The obtained data show that salinity stress decreased significantly photosynthetic pigments compared with control plants (Fig 1). Different investigators confirmed the reduced effect of salinity on photosynthetic pigments of sunflower[39] and faba bean plant[3&40]. These decreases in photosynthetic pigments by sea water stress could be due to the degradation or inhibition in chlorophyll biosynthesis[41] and/or chloroplasts disorganization[42]. Moreover, the decreases in chlorophyll content under salinity stress could be caused by chlorophylase enhanced activity, pigment protein complex instability and the disruption in fine structure of the chloroplast[43]. Whereas, cyanobacteria addition to soil combined with different NPK rates (R & ½R NPK) under normal and diluted sea water stress caused an enhancement effect on photosynthetic pigments of wheat plant (Fig 1). These results of cyanobacteria enhancing role are in agreement with those obtained[44&45]. The enhancing role of cyanobacteria amended to soil might be due to its important role on increasing the availability of water and minerals to plant cells[44]. Soaking wheat grains with GB increased photosynyhetic pigments in wheat plant relative to control plants under normal, diluted sea water either in soil amended with cyanobacteria or in absence of cyanobacteria, recommended or half recommended dose of NPK. These promotive effect on

photosynthetic pigments under normal and salinity stress might be due to its role in preventing photoinhibition[46]. In addition, electron flow via thylakoid membrane maintenance, protection of lipids of photosynthetic apparatus and Rubisco enzymes thus maintaining efficiency of photosynthesis[47&48].

Regarding to the effect of different treatments on total soluble sugars of wheat plant under sea water stress, in the present study, our results demonstrated that, irrigation of wheat plant with diluted sea water (7.69 EC dS/m) increased total soluble sugars in the tested cultivar (Giza 168) of wheat plant (Fig 2). Our obtained results are in good agreement with those obtained on sunflower plant[49] and flax cultivars[5]. Accumulation of soluble carbohydrate play a key role in alleviating salinity stress, either via osmotic adjustment or by conferring some desiccation resistance to plant cells[49]. The accumulation of organic solutes especially sugars are the main solutes involved in osmotic adjustment in glycophytic plants submitted to osmotic and saline stress[50]. Moreover, cultivation of wheat plant in the presence of cyanobacteria led to marked increases in soluble sugars when compared with plants cultivated without cyanobacteria, which stimulated the accumulation of total soluble carbohydrates in salt stressed wheat plant either via increasing endogenous levels of certain phytohormones or by acting as activators of carbohydrates synthesis. Similar results to ours reported here were obtained[51]. they found that cyanobacteria are generally considered tolerant to salt stress, as they are found to soils. Data obtained revealed also, that treatment of wheat plant with 5 mg/l GB stimulated the accumulation of total soluble sugar as compared with the corresponding salinity levels in the presence or absence of cyanobacteria. These results are in good agreement with the results observed[36,37&52] on canola and sunflower plants treated with glycinebetaine**.** The effects of glycinebetaine on accumulation of soluble sugars probably, attributed to the protective effects of GB on the photosythetic systems. GB, also, plays a protective role in salinity tolerance by maintenance of the redox status[36].

Data presented in Fig 3 shows that, salinity stress caused significant increases in free amino acids and proline contents of wheat plants as compared with control plant. These results are in good agreement with the results observed on sunflower plant[49] faba bean[3] and flax plant[**43**]**.** They concluded that salinity stress was capable of acting as activators of free amino acids accumulation. It is clearly that accumulation of amino acids in wheat plant exposed to salt stress may be attributed to the disturbance in amino acid metabolism. Osmolytes such as (proline) are known to play an important role in protecting macromolecules by stabilizing protein structure and/or scavenging ROS produced under stress conditions[53]. It is also involved in cell osmoregulation, protection of proteins during dehydration and can act as an enzymatic regular during stress conditions[54&55]. It was also, reported that proline act as free radical scavengers and/or enzyme protectants as well as compatible solutes[56]. Many functions have been postulated for, proline and free amino acids as could be protective agents of enzymes and membranes[57]. Cyanobacteria significantly increased, free amino acids content and proline of wheat plant. It was observed culture cyanobacteria capable of enhancing plant growth and observed the presence of extracellular proteins in the range of 32–82 $\mu g/ml^{-1}$ and an array of amino acids. Furthermore, GB significantly enhanced the stimulatory role of salt stress on the production of free amino acid and proline in wheat plant in the presence and absence of cyanobacteria. Similar results have been obtained[36&37]. These accumulation of amino acids in wheat plant probably attributed to the disturbance in amino acid metabolism. The higher level of proline content in wheat shoots may be due to expression of gene encoding key enzymes of proline synthesis and low activity of the oxidizing enzymes which is controlled by osmotic and salinity stress[50].

Data presented in Tables (3&4) and Fig (4) revealed the effect of salinity water and glycine beataine in absence or presence of cyanobacteria under the recommended or half recommended doses of NPK on yield attributes as well as nutritional values of wheat plant. Irrigation of wheat plant with diluted sea water (7.69 dS/m EC) reduced yield attributes (plant height, spikes number/plant, spike length, spikes wt/plant, grains wt/plant and 1000 grains/plant) as well as carbohydrate%, protein%, N, P, and K percentages of the yielded grains compared with control plant. These results are in good agreement with those on sunflower[8], wheat plant[7] and sunflower cultivars[37]. These reductions resulted from the reductions in morphological parameters, photosynthetic pigments and the disturbance in mineral uptake of wheat plant. 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[40,59&60]. Meanwhile, cultivation of wheat plant in soils amended with cyanobacteria caused significant increases in yield attributes compared with those cultivated in absence of cyanobacteria. In the present study, wheat yield had significantly improved in the saline soil (EC 7.96 dSm/m) and were able to withstand the salt stress in presence of cyanobacteria in the recommended and half recommended doses of NPK. In this context, it was revealed that wheat and rice yields (straw and grain) along with carbohydrate%, protein% as well as macronutrients percentages (N, P and K) had increased significantly

in response to cyanobacteria under saline soil[61&44]. It was indicated that cyanobacteria have the ability to fix the atmospheric nitrogen[62] and possess some soil phosphate solubilizing species that solubilize the insoluble phosphate through excreting for organic acids that solve the common problem of P chemical fixation in all types of soils[63], thus both N and P become available to plants sharing in the increase of both straw and grains yield. Regarding the promotive effect of glycinebetaine on yield attributes and biochemical constituents of the yielded grains, glycinebetaine (GB) promotes plant growth and yield under normal or stress conditions due to its osmoprotective effect on photosynthetic machinery and regulation of ion homeostasis[64] as well as improving CO_2 assimilation in plants under salinity stress[65] and because of its role in biosynthesis and transport of growth regulators like cytokinins that may have a role in the transport of photoassimilates[66].

We can also figure out, that application cyanobacteria, and/or GB plays a protective role in salinity tolerance by increasing some photosynthetic pigments and osmoprotective compounds thus lead to improve growth and yield quantity and quality of wheat plant.

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