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Role of Blue-Green Algae, Glutathione and Salicylic Acid on The Oxidative Defense Systems of Wheat Plant Grown in saline soil

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Abstract: Cyanobacteria, known as blue-green algae are prominent inhabitants of many agricultural soils, where they potentially contribute to improve soil fertility and crop productivity. Therefore, it becomes important to consider how glutathione and salicylic acid mitigate the oxidative salt stress in the presence or absence of cyanobacteria and recommended or half recommended doses of NPK experienced by wheat cultivar sakha 93. It was observed herein that, salt stress elicit an effect on total soluble sugars, free amino acids. The levels of antioxidant compounds (ascorbic acid and proline) were changed in response to salt stress. Soaking wheat plant with glutathione and salicylic acid improve vegetative plant resistance in the presence of cyanobacteria probably by increasing levels of antioxdant substances and enhancing the activities of antioxidant enzymes (superoxide dismutase, catalase; peroxidase, glutathione reductase as well as ascorbate peroxidase).

Keywords : Antioxidants, Blue-green algae, Glutathione, Salicylic acid, Salinity, Wheat

Introduction

Salt stress is considered as one of the most important abiotic factors limiting plant growth and productivity. Salt tolerance mechanisms are quite complex, involving osmotic adjustment, compartmentation of toxic ions[1]**.** Metabolite accumulation, ion homeostasis, redox control and scavenging activated oxygen species (ROS)[2]. Salinity produces oxidative stress in plant tissues[3]**.** This stimulates the generation of active oxygen species. Reactive oxygen species affect lipid peroxidation, protein denaturation and DNA mutation[4]. Several enzymes are involved in the detoxification of ROS, which resulted under salinity stress. Superoxide dismutase (SOD) is the first defense enzyme which converts superoxide to H_2O_2 and this, can be scavenged by catalase (CAT) and different classes of peroxides (POD)[5]. To remove reactive oxygen species, plant cells possess an antioxidant system consisting of low molecular- weight antioxidants, such as ascorbate, α- tocopherol, glutathione and carotenoids, as well as antioxidant enzymes.

Glutathione is a tripeptide (α-glutamyle cysteinyl glycine), which has been detected virtually in all cell compartments[6]. Glutathione, GSH plays an important role in the response of plants to environmental stresses,

including oxidative stress due to the generation of active oxygen species; improve seed germination and seedling growth under salt stress[7]. GSH may also be involved in the redox regulation of the cell cycle[8]. It is involved in the ascorbate / glutatione cycle and in the regulation of protein thiol – disulphid redox status of plants in response to abiotic and biotic stress[9].

Salicylic acid is an endogenous growth regulator and acts as non-enzymatic antioxidant of phenolic nature, which participates in the regulation of physiological processes in plants. Its role in the defense mechanisms against biotic and abiotic stress has been well documented[10]. Salicylic acid was found to cause an inhibition of catalase and H_2 O_2 scavenging enzyme. SA plays an essential role in the regulation of ROS and antioxidant enzymes[11].

Cyanobacteria, known as blue-green algae, are oxygenic, photosynthetic prokaryotic organisms[12]**.** It is hypothesized that the colonization by cyanobacteria leads to the release of a diverse array of biologically active metabolitesin the rhizosphere, which may elicit induced systemic responses in plant, thereby enhancing plant growth under stress conditions[13,14]. Mechanism of any adaptation in cyanobacteria has been elucidated in terms of osmoprotective compounds and maintenance of low internal contents of inorganic ions. These substances even in high concentration are compatible with cellular metabolism, and is assumed that they are able to protect macromolecules against denaturation and thus to improve their function in cell environment of stress[15]. Cyanobacteria are prominent inhabitants of many agricultural soils where they potentially contribute towards biological nitrogen fixation, help in phosphate solubilization and mineral release to improve soil fertility and crop productivity[16]. 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[17].

In the present study, we report the impact of cyanobacterial colonization, with or without glutathione and salicylic acid on metabolic (total soluble sugar, free amino acid, proline and ascorbic acid) and enzymatic (superoxide dismutase, catalase, peroxidase, ascorbate peroxidase and glutathione reductase) status of wheat plant in salt stressed soil. 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 field experiment was carried out at the Agricultural Station of Faculty of Agricultural, Fayoum University, Fayoum Governorate–Egypt, during two successive winter seasons of 2012/2013 and 2013/2014. Grains of wheat cultivar (sakha 93) were obtained from Agricultural Research Centre Assuit branch, Egypt. Cyanobacteria were obtained from the Microbiology Department, Soils, Water and Environment Res. Inst., Agric. Res., Centre. Chemical compounds (glutathione, GHS and salicylic acid, SA) were supplied from SIGMA – ALDRICH Company. Two experimental sites were chosen with physical and chemical analysis as shown in Table (1). Soil analysis was carried out according to[18,19].

Table 1. Physical and chemical analysis of two sites used before planting cultivated soils of two different sites with soil salinity levels of 6.25 and 9.38 dS m^{-1} at the experimental farm of the Faculty of Agriculture, Fayoum University, Egypt.

Property	**site 1**	**site 2**
Clay %	27.25	26.50
Silt %	21.75	22.00
Sand %	51.00	51.50
Soil texture:	**Sandy clay loam**	
Chemical		
pH	7.89	8.07
EC (dSm−1)	6.30	9.42
Organic matt	0.30	0.86
CaCO3 %	9.56	7.84
Some available macro- and micronutrients (ppm)		
N	206.00	195.00
P	24.69	18.00
K	284.00	280.00
Fe	41.16	46.92
Mn	23.00	22.26
Zn	0.36	0.31
Cu	0.20	0.24

The aim of this work was to investigate the effect of cyanobacterial addition to the soil and/or soaking treatment with glutathione (GHS) and salicylic acid (SA) at concentration 10 mg/l for 12 hours of wheat grains (Sakha 93) cultivar grown under two environmental salty soil conditions with recommended (R) or half recommended (½R) dose of nitrogen, phosphorus and potassium (NPK) fertilizer.

The experimental design was split-split plot design with four replicates. The two experimental sites occupy the main plots, while nitrogen, phosphorus and potassium (NPK) fertilizer treatments were randomly assigned in sub plots and the treatments of (cyanobacteria, glutathione and salicylic acid) were allocated at random in sub-sub plots. Grains of wheat were sown at the 15 November in both seasons in rows 4 meters long, the distance between rows was 25 cm, plot area was 12 m (3.0 m in width and 4.0 m in Length).

The recommended agricultural practices of growing wheat grains were applied; the seeding rate was (60 Kg grains/Fed). Pre-sowing, 150 kg/fed. of calcium super-phosphate (15.5 % P_2O_5) was applied to the soil. The recommended (R) or half recommended (½R) dose of (NPK) was applied after emergence in the form of ammonium nitrate 33.5% at rate of 100 Kg/fed was applied at five equal doses before the 1st, 2nd, 3rd, 4th and 5th irrigation. Potassium sulfate (48.52 % K_2O) was added at two equal doses of 50 kg/fed, before the 1st and 5th irrigations. Irrigation was carried out using the new sprinkler irrigation system where water was added every 7 days. Plant samples were taken for chemical analysis after 75 days from sowing for chemical analysis of total soluble sugars, proline, free amino acids, lipid peroxidation, Assay non enzymatic antioxidants ascorbate, oxidative enzymes activities (superoxide dismutase SOD, catalase CAT, peroxidase POX , ascorbate peroxidase APX and glutathione reductase GR).

Chemical analysis:

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 by[20]. 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% H_2SO_4) in boiling water bath for ten minutes and reading the cooled samples at 625 nm using Spekol Spectrocololourimeter VEB Carl Zeiss[21].

Free amino acids

Free amino acid content was extracted according to the method described by[22]. Free amino acid was determined with the ninhydrin reagent method [23]. 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 described by[24]. Two ml of proline extract, 2.0ml of acid ninhydrin and 2.0ml 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.

Lipid peroxidation

Lipid peroxidation was determined by measuring the amount of produced Malondialdehyde (MDA) by the thiobarbituric acid (TBA) reaction as described by[25]. The crude extract was mixed with the same volume of a 0.5 % (w/v) TBA solution containing 20 % (w/v) tricholoroacetic acid (TCA). The mixture was heated at 95 _C for 30 min and then quickly cooled in an ice-bath. The mixture was centrifuged at 3,0009g for 10 min and the absorbance of the supernatant was monitored at 532 and 600 nm. After subtracting the non-specific absorbance (600 nm), the MDA content was determined by its molar extinction coefficient (155 mM-1 cm-1).

Assay non Enzymatic Antioxidants

Ascorbate

The water soluble ascorbic acid (ASC) was determined by the methods of[26]. Total ASC was determined in a reaction mixture of 1.0 ml containing 50 µl apoplastic fluid, 100 µl phosphate buffer 0.2 m (pH 7.4), 50 µl dithiothreitol (DDT) 10 mm. After shaking and incubation for 20 min at 42 ºC in water bath, 50 µl of 0.5 % N-ethylmaleimide (NEM) were added, with a following incubation for 1 min at room temperature. ASC levels were determined in the same way with exception for the increasing of DDT and NEM which were substituted by water. Colouring was obtained by adding of 250 µl 10 % trichloroacetic acid, 200 µl of 42 % phosphoric acid, 200 µl of dipiridil dissolved in 70% ethanol and 100 µl of 3% phosphoric acid. After shaking, a mixture was incubated for 40 min at 42ºC and the absorbance values of the final coloured solutions were recorded at 525 nm.

Assay of enzymes activities

Enzyme extracts were collected following the method described by[27]. Leaf tissues were homogenized in ice-cold phosphate buffer (50 mM, pH 7.8), followed by centrifugation at 8,000 rpm and 4-C for 15 min. The supernatant was used immediately to determine the activities of enzymes.

Peroxidase

Peroxidase (POX, EC 1.11.1.7) activity was assayed by the method of[28]. The reaction mixture used for estimating POX contained 2 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H_2O_2 and 0.5 ml of the enzyme extract. The solution was incubated for 5 min at 25 ^oC after which the reaction was terminated by adding 1 ml of 2.5 N H_2SO_4. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a reagent blank prepared by adding the extract after the addition of 2.5 N H_2SO_4 at the zero time.

Superoxide dismutase

Superoxide dismutase (SOD, EC 1.12.1.1) activity was spectrophotometrically assayed at 560 nm by nitro-blue-tetrazolium(NBT) reduction method[27]. The reaction mixture (3 ml) contained 150 µ riboflavin (13µM), 2.5 ml methionine (13µM), 250 µ NBT (63 µM), 50 µphosphate buffer (50 mM, pH 7.8) and 50 µ

enzyme extract. One unit of SOD activity was defined as the amount of enzyme protein required for inhibition of the 50 % reduction of NBT.

Catalase

Catalase (CAT, EC 1.11.1.6) activity was determined spectrophotometrically by following the decrease in absorbance at 240 nm[27]. The mixture (3 ml) contained 1.9 ml phosphate buffer (50 mM, pH7.0), 100 µl enzyme extract and 1 ml 0.3 % H_2O_2. The reaction was initiated by adding enzyme extract. One unit of CAT activity was defined as the 0.01 deduction in absorbance at 240 nm per minute. The enzyme activities were calculated by[29].

Ascorbate peroxidase

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined as described by[30]. The reaction mixture (1 ml) contained 50 mM potassium phosphate (pH 7.0), 1.2% sucrose, 1 mM hydrogen peroxide, 0.5 mM ascorbate and 10 mM 3- aminotriazole (an inhibitor of catalase). The hydrogen peroxide-dependent oxidation of ascorbate was followed by monitoring the decrease in absorbance at 290 nm, using the extinction coefficient of 2.8 mM cm^{-1}.

Glutathione reductase

Glutathione reductase (GR, EC 1.6. 4.2) activity was determined by the oxidation of NADPH at 340 nm with the extinction coefficient of 6.2 mM cm^{-1} as described by[31]. The reaction mixture was composed of 100 mM potassium phosphate buffer (pH 7.8), 2 mM EDTA, 0.2 mM NADPH, 0.5 mM glutathione (oxidizied form, GSSG) with an appropriate volume of enzyme extract in a 1 ml volume. The reaction was initiated by the addition of NADPH at 25 °C.

Statistical analysis

The data were statistically analyzed on complete randomized design system according to[32]. Combined analysis of the two growing seasons was carried out. Means were compared by using least significant difference (LSD) at 5% levels of probability.

Results

Total soluble sugars

The effect of glutathione, salicylic acid on wheat plant (sakha 93) cultivated in soil amended with cyanobacteria, and recommended or half recommended doses of NPK on the total soluble sugar are shown in Fig 1. Increasing salinity levels from 6.26 to 9.39 EC dS m^{-1} significantly increased total soluble sugars in wheat plant in recommended and half recommended dose of NPK. Moreover, cultivation of wheat plant in the presence of cyanobacteria led to marked increase in soluble sugars when compared with plants cultivated without cyanobacteria with or without GSH, and SA.

Data obtained revealed also, that soaking wheat plant with 10 mg/l GSH or SA stimulated the accumulation of total soluble sugar as compared with the corresponding salinity levels in the presence or absence of cyanobacteria. More pronounced increase in total soluble sugars was obtained with plants treated with salicylic acid (Fig. 1) in the high salt concentration. The maximum increases in total soluble sugars were obtained by SA in soil amended with cyanobacteria and R dose of NPK over the all other treatments.

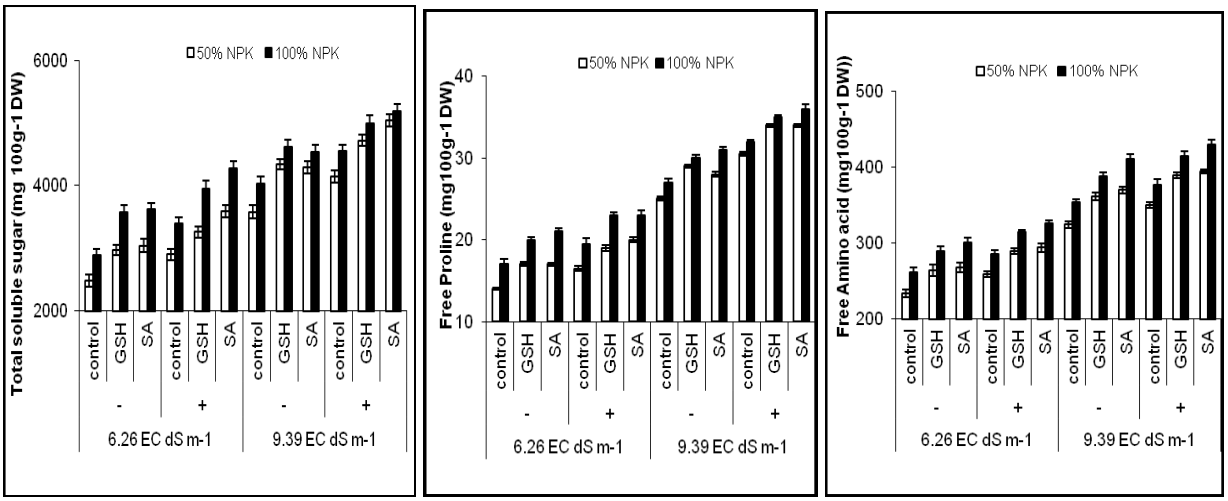
Data clearly show that, cyanobacteria + 1/2 R dose of NPK treatment caused non significant increases in TSS of wheat pant as compared with the control plants with R doses of NPK and significant increases with, cyanobacteria + 1/2 R dose of NPK with GHS or SA in both salinity site.

Amino acids and Proline

Data presented in Fig 1, show that salinity stress caused significant gradual increases in free amino acids and proline content with increasing salinity levels from 6.26 to 9.39 EC dS m^{-1}. Furthermore, GSH and SA 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 highest level of amino acids and proline was found in plants pretreated with SA in the highest level of salt stress as compared to the corresponding control plant. The maximum increases in both free amino acides and proline were obtained by using the triple treatment (cyanobacteria + SA + R dose of NPK) over the all other treatments.

Data clearly show that, cyanobacteria with GHS or SA + 1/2 R dose of NPK treatment caused more pronounced increase in free amino acid and proline of wheat pant as compared with the corresponding control plants with R doses of NPK.

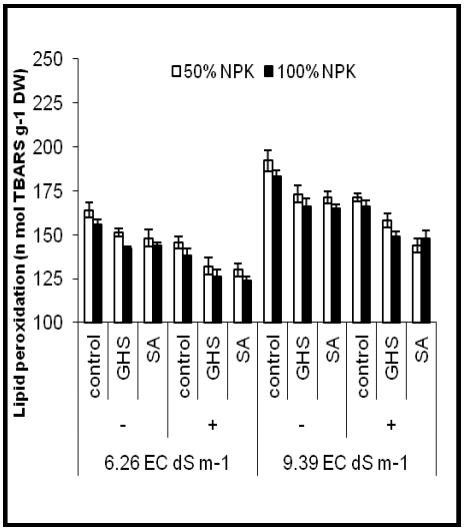


LSD at 5% 260 **LSD at 5% 2.0** **LSD at 5% 21**

Fig .1 Effect of glutathione and salicylic acid (10 mg/l) in absence (-) or presence (+) of cyanobacteria on TSS, free amino acid and proline (mg/ 100g dry weight) of wheat plant in saline soil (at 75 days from sowing) Each value represents the mean ± standard error (n =3).*GSH = Glutathione, *SA= Salicylic acid.

Lipid peroxidation

High salt in plant induced oxidative damage, assessed by measuring the levels of malondialdehyde (MDA). The effect of salt was more pronounced by significantly increase in lipid peroxidation reaching the maximum value at 9.39 EC dS m^{-1} salinity level in the tested wheat (Fig. 2). The values of peroxidation products positively related to the concentration of salt in the soil, thereby there is a positive relation among the amount of lipid peroxidation products and the degree of membrane damages resulted from the injurious salt stress. Moreover, decreases in lipid peroxidation by GSH and SA acid in the absence and presence of cyanobacteria in the two salinity levels as compared with the corresponding control. Data in Fig. 2 showed that amended soil with cyanobacteria caused significant decreases in lipid peroxidation the percentage of decrease was (11.3% and 11.6%) in salinity level 6.26 EC dS m^{-1} and (10.9% and 9.3%) in salinity level 9.39 EC dS m^{-1} in R and ½ R dose of NPK respectively as compared with the corresponding untreated soil with cyanobacteria. Moreover there is no significant decrease in lipid peroxidation between R and ½ R dose of NPK in all treatments as compared with the corresponding control.



LSD at 5% 1.2

Fig. 2 Effect of glutathione and salicylic acid (10 mg/l) in absence (-) or presence (+) of cyanobacteria on Lipid peroxidation (n mol TBARS /g dry weight) of wheat plant under saline+ soil (at 75 days from sowing) each value represents the mean ± standard error (n =3).
***GSH = Glutathione *SA= Salicylic acid.**

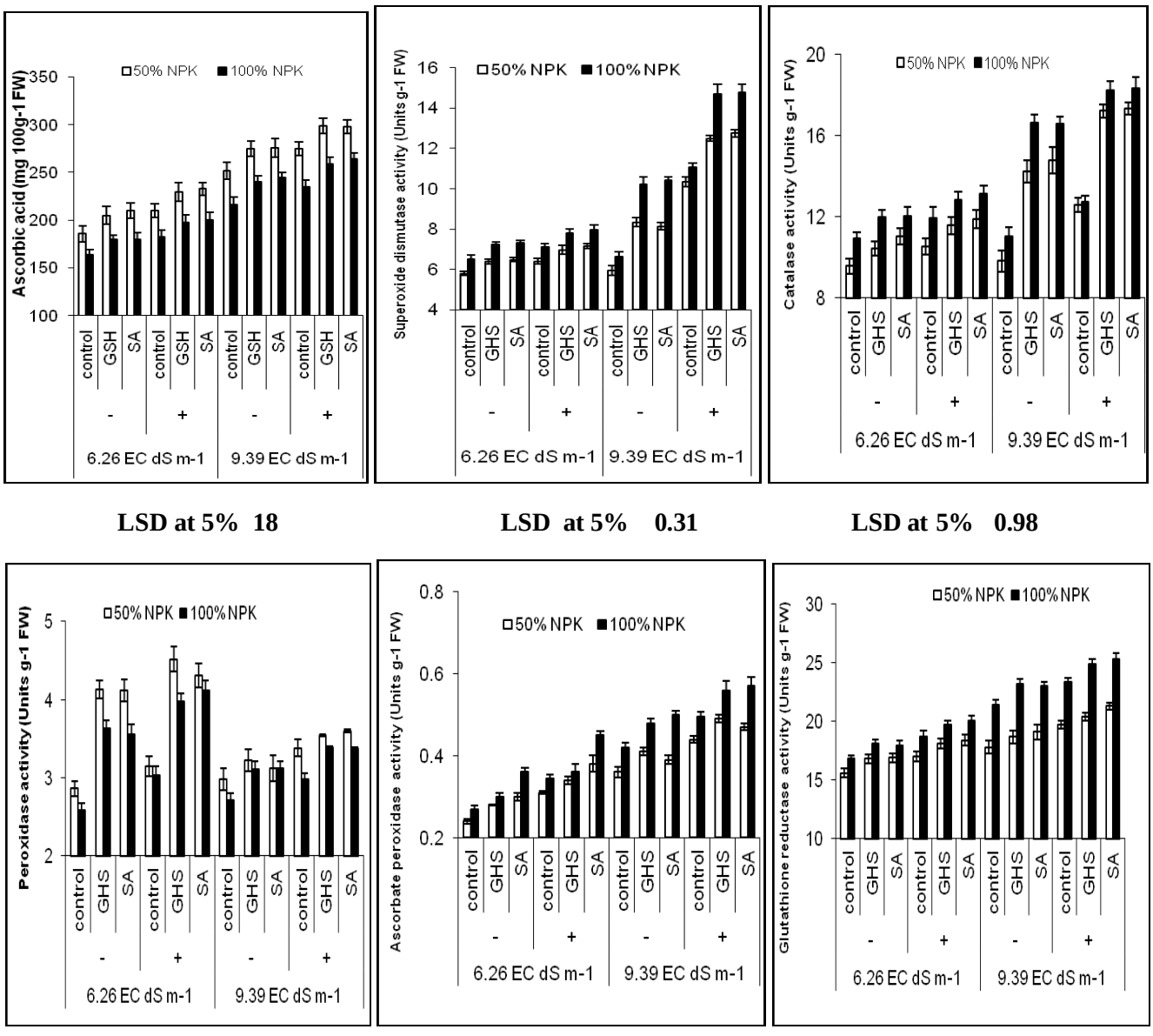
Changes in ascorbic acid

Data show that salinity stress caused significant gradual increase in ascorbic acid content with increasing in salinity levels from 6.26 to 9.39 EC dS m^{-1} in both treatment with R and ½ R of NPK. Marked increases in ascorbic acid were observed in wheat plants inoculated with cyanobacteria, glutathione and salicylic acid in both sites of salinity (Fig 3). Moreover, there was significant increase in ascorbic acid in ½ R dose of NPK as compared with the corresponding treatment in R dose in all treatment (cyanobacteria, glutathione and salicylic acid). The most effective treatment was cyanobacteria with GHS or SA + 1/2 R dose of NPK treatment in the salinity level 9.39 EC dS m^{-} of wheat pant . The increase in endogenous ascorbic acid was about 1.5 folds in 9.39 EC dS m^{-1}compared to 6.26 EC dS m^{-1} stressed plants.

Antioxidant enzymes activity

Plants have evolved efficient systems for ROS removal, which include specific ROS- scavenging enzymes like SOD, APX, CAT, and GR. Results in this study showed that SOD and CAT activity in NaCl - stressed plants was significantly superior in the salinity level 9.39 EC dS m^{-1} than in the salinity level 6.26 EC dS m^{-} as compared with the corresponding treatment(Fig 3). Our results demonstrated also, that peroxidase POX activity was significantly decreased with increasing stress conditions in both ½ R and R dose of NPK in the plants treated with GHS or SA (Fig 3). However, treating of salts stressed plants with GSH or SA promoted the activities of POX in the absence and presence of cyanobacteria (Fig 3). The activity of APX and GR in wheat plant was positively related to the dose of salinity. Application of cyanobacteria, and/or GSH or SA in general, significantly increased the activity of APX and GR in stressed plants. The most pronounced increase was observed with SA or GSH in both salinity sites and R dose of NPK as compared the corresponding salinity level.

We can also figure out, that application of cyanobacteria, and/or GSH or SA, play a protective role in salinity tolerance by increased the activities of the antioxidant substances as well as antioxidant enzymes. Cyanobacteria, and/or GSH or SA act at the cellular level as affects on the redox status of the cell.



LSD at 5% 18 **LSD at 5% 0.31** **LSD at 5% 0.98**

LSD at 5% 0.26 **LSD at 5% 0.03** **LSD at 5% 1.2**

Fig. 3 Effect of glutathione and salicylic acid (10 mg/l) in absence (-) or presence (+) of cyanobacteria on ascorbic acid (mg /100g fresh weight) and antioxidant enzyme activities of wheat plant in saline soil (at 75 days from sowing) each value represents the mean ± standard error (n =3).
***GSH = Glutathione, *SA= Salicylic acid.**

Discussion

Total soluble sugars

In the present study, our results demonstrated that the increasing salinity levels up to 9.39 EC dS m^{-1} increased total soluble sugars in the tested cultivar (Sakha 93) of wheat plant. Our obtained results are in good agreement with those obtained by[33] on sunflower plant. Moreover, 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[34]. The accumulation of organic solutes especially sugars are the main solutes involved in osmotic adjustment in glycophytic plants submitted to osmotic and saline stress[35]. Moreover, cultivation of wheat plant in the presence of cyanobacteria led to marked increase 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 in were obtained by[36] 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 10 mg/l GSH or SA 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 by[37] on canola plant treated with glutathione**.** The effects of glutathione on accumulation of soluble sugars probably, attributed to the protective effects of glutathione on the photosythetic systems. GSH, also, plays a protective role in salinity tolerance by maintenance of the redox status[38]. The total soluble sugar increased as a result of SA treatment and this means that the osmotic adjustment was associated with an increase in total soluble sugars**.** These results are in good agreement with those obtained by[39] who, reported that, SA increases of all soluble carbohydrate in the shoot during drought stress is effective on the balance against osmotic pressure. Moreover, [40] demonstrated that salicylic acid, urges the leaves of *Lycopersicon esculentum* Mill. L. to fill up more soluble sugar and increases photosynthetic carbohydrate is a signal for water deficiency tolerance. The high carbohydrate concentration with its role to reduce water potential helps to prevent oxidative losses and protein structure maintenance during water shortage. Also carbohydrates play a molecule role for sugar responsible genes that give different physiological response like defensive response and cellular expansion[41].

Amino acids and Proline

Data presented in Fig 1, shows that salinity stress caused a significant gradual increases in free amino acids and proline contents with increasing in salinity levels from 6.26 to 9.39 EC dS m^{-1}. These results are in good agreement with the results observed by[33] on sunflower plant**.** 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[42]. It is also involved in cell osmoregulation, protection of proteins during dehydration and can act as an enzymatic regular during stress conditions[43].

Cyanobacteria significantly increased, free amino acids content and proline of wheat plant Sakha 93. [44]Karthikeyan N et al observed culture cyanobacteria capable of enhancing plant growth and observed the presence of extracellular proteins in the range of 32–82 µg ml^{-1} and an array of amino acids. Furthermore, GSH and SA 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 by[45,46]. Proline accumulation by salicylic acid and glutathione treatments increased in different plant species, under oxidative stresses[37,47]. The accumulation of amino acids in wheat plant exposed to stress probably attributed to the disturbance in amino acid metabolism[37]. The higher level of proline content in canola 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[35]. It was also, reported that proline act as free radical scavengers and/or enzyme protectants as well as compatible solutes[48]. Many functions have been postulated for, proline and free amino acids as could be protective agents of enzymes and membranes[49].

Lipid peroxidation

High salt in plant induced oxidative damage, assessed by measuring the levels of malondialdehyde (MDA). The effect of salt was more pronounced by significantly increase in lipid peroxidation reaching the maximum value at 9.39 EC dS m^{-1} salinity level in the tested wheat. The values of peroxidation products positively related to the concentration of NaCl in the soil, thereby there is a positive relation among the amount of lipid peroxidation products and the degree of membrane damages resulted from the injurious salt stress. These increases may be attributed to salinity effect which could modify the membrane structure and stimulate O_2 production, which facilitates lipid peroxidation[50]. In addition, the presence of oxidation products such as MDA in biological systems is also, related to the beginning of peroxidation of unsaturated fatty acids. During Lipid peroxidation the double bonds of unsaturated fatty acids leading to diene conjugation. The increase in lipid peroxidation may be due to the incapability of antioxidants to neutralize and scavenge all the active oxygen species resulted from salt stress. The present results are in agreement with the results of Chaparzadeh N et al[38].

Cyanobacteria decreased lipid peroxidation of wheat plant (Sakha 93) when compared with the untreated plants. Glutathione is a water soluble antioxidant which reacts directly or indirectly with the reactive oxygen species so, reduces stress injurious effects on membrane. Moreover, decreases in lipid peroxidation by GSH and SA in the presence of cyanobacteria, may be also due to their effects on the activities of antioxidant enzymes and/or the high level of the endogenous GSH and SA (Fig 2). Glutathione may mitigate the deleterious effect of salt via protection of membrane[51]. Stabilization of membrane permeability by glutathione may reduce passive Na+ influx and thus enhance plant salt tolerance. This may occur through lipid peroxidation prevention and maintenance of cellular redox balance[52]. In response to SA pretreatment, [53] Delavari PM et al found that on Ocimum basilicum plants sprayed with SA led to a significant decrease in the level of lipid peroxidation. [54] reported that the application of SA decreased MDA and total antioxidant activity under salinity and drought stresses.

Ascorbic acid

Salt stress accelerates the formation of active oxygen species. The lifetime of active oxygen species within the cellular environment is determined by the antioxidant system, which provides crucial protection against oxidative damage. The antioxidant system comprises numerous compounds of low molecular weights and enzymes[2]. The level of endogenous total ascorbic acid was significantly increased in the stressed wheat plant. The increases in endogenous ascorbic acid in stressed plants were also, observed by Sairam RK et al [5].

Marked increases in ascorbic acid were observed in wheat plants inoculated with cyanobacteria, glutathione and salicylic acid (see Fig 3). Reduced ascorbate, are the major water soluble antioxidants which scavenging reactive oxygen species to maintain the integrity of cell structures and the proper functions of various metabolic pathways[55]. Recently, much attention is given for cyanobacteria due to the presence of biologically active compounds such as phycobilins, phenols, antioxidants, terpenoids, steroids and polysaccharides[56]. [57] Sharathchandra K et al revealed the utilization of certain strains of cyanobacteria for the production of biologically active compounds particularly, antioxidants. [58] observed that when SA was applied exogenously at suitable concentrations, enhance the efficiency of antioxidant system in plants.

Antioxidant enzyme activities

Plants have evolved efficient systems for ROS removal, which include specific ROS- scavenging enzymes like SOD, APX, CAT, and GR. These enzymes are observed to act as ROS scavenger[59]. The phospholipids membranes are impermeable to charged $\hat{O}^2$ molecules therefore superoxide dismutase are present for the removal of $\hat{O}^2$ in the compartments where $\hat{O}^2$ radicals are formed[60]. SOD that acts on first product of free radical generation (O^{2-}) showed relatively higher activities. Results in our study showed that SOD and CAT activity in $NaCl^-$ stressed plants was significantly superior (see Fig 3). Hydrogen peroxide is scavenged by catalase CAT, breaking it directly to form water and oxygen and an increase in its activity that increase in stress tolerance. Exogenous application of all the tested materials cyanobacteria, glutathione and SA markedly enhanced SOD and CAT activity than that of corresponding control plants. Some researchers also, suggest that salt stress leads to a decrease in SOD activity in salt-sensitive plants but to an increase in salt-tolerance one[48].

Our results demonstrated also, that POX activity was significantly decreased with increasing stress conditions (Fig 3), which are supported by the findings of [61]. The reductions in peroxidase activities suggest that these enzyme were unable to completely neutralize H_2O_2 resulted from the oxidative salt stress [37]. However, treating of salts stress plants with cyanobacteria, GSH or SA promoted the activities of POX (Fig 3). The activities of various antioxidant enzymes (CAT, POX and SOD) were increased with a concomitant increase in proline content as a result of salinity exposure and/or SA treatment, thereby providing enhanced tolerance against salinity stress [62]. The possible explanation of involvement of SA in ROS regulation under saline condition could be an up-regulation of amplification loops involving NADPH oxidases in ROS signaling in several studies[63]. These loops might be activated by low levels of ROS and result in enhanced production and amplification of the ROS signals in specific cellular locations for the homeostasis of cellular redox[63]**.** [64]reported the exogenous application of salicylic acid enhanced the activities of antioxidant enzymes APX and SOD with a concomitant decline in the activity of CAT in maize plants.

The ascorbate pool can be reduced by oxidative stress when regeneration capacity is over come[38]. The activity of ascorbate peroxidase and Glutathione reductase in wheat plant positively related to the dosage of NaCl. The results are in good agreement with those obtained by Sairam RK et al[5].

Application of cyanobacteria, and/or GSH and SA in general, significantly decreased lipid peroxidation activity in Sakha 93, which was increased with the corresponding salinity levels. Also, the studed treatments significantly increased the activity of APX and GR in stressed plants. Similar results suggest that the increase in the activities of antioxidant enzymes is not a direct consequence of H_2O_2 accumulation, but is mediated through GSH in intracellular environment of L. latifolium[65]. Moreover, [66] Erasalan F et al reported that, exogenous SA could regulate the activities of antioxidant enzymes and increase plant tolerance to abiotic stresses. In our study, salt stress could increase the GR activity to a small extent. However, when SA treated seeds were subjected to salt stress the activity markedly increased which rendered rapid recycling of GSH in line with better synthesis of GSH under salt stress conditions (Fig. 3). The role of SA in enhancing the activity of GR was reported by many plant studies[67]. Moreover, we reported the correlation between enhanced GR activity and better GSH levels as well as abiotic stress tolerance including salinity[68]. [69] Singh PD et al illustrated that cyanobacterial inoculation evoked systemic accumulation of biochemical substances, which induced levels of phenylpropanoids and phytohormones and enhanced enzymatic profile in rice leaves[70].

We can also figure out that application of cyanobacteria, and/or GSH and SA, play a protective role in salinity tolerance by increased the activities of the antioxidant substances as wells as antioxidant enzymes. cyanobacteria, and/or GSH and SA acts at the cellular level as affects the redox status of the cell. The results suggested that 1/2 of the recommended dose of NPK fertilizer could be saved by using of cyanobacteria. Also it emphasized the prospects and potentials of using cyanobacteria biofertilizers as renewable natural nitrogen resources for wheat and cyanobacteria help to increase the oxidative defense system of wheat. Cynobacteria is also none polluting, inexpensive and utilize renewable resources.

Abbreviations

ASC	Ascorbic acid
CAT	Catalase
GHS	Glutathione
H_2O_2	Hydrogen peroxide
MDA	Malondialdehyde
POX	Peroxidase
PPO	Polyphenol oxidase
ROS	Reactive oxygen species
SA	Salicylic acid
SOD	Superoxide dismutase

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