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ROLE OF ENZYMATIC AND NON ENZYMATIC ANTIOXIDANT IN AMELIORATING SALINITY INDUCED DAMAGE IN NOSTOC MUSCORUM

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Abstract

Presence of high salt concentration in the growth medium adversely affected the plant growth and productivity by altering its metabolic activities. Experiments were conducted on cyanobacteriaum *Nostoc muscorum* grown in nitrogen free medium supplemented with 250 m*M* NaCl to evaluate the salt stress induced changes in growth, antioxidants and lipid composition. Salt stress significantly reduced the growth and physio-biochemical attributes. Salt stress increased malonaldehyde content thereby causing alterations in the lipid fraction. Significant reduction in polyunsaturated fatty acids including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI) and phosphatidylserine (PS) was observed. Where as diacylglycerol, sterol ester and non-esterified fatty acids were increased. Activities of antioxidant enzymes and contents of non-enzymatic antioxidants including glutathione enhanced due to salt stress. An increase in accumulation of proline was also observed. Hence increased activity of antioxidants and altered fatty acid composition was observed in salt stressed *Nostoc muscorum*.

Key words: *Nostoc muscorum*, Antioxidants, Proline, Lipids, MDA, Salt stress.

Introduction

Sessile organisms including lower as well as higher plants are often confronted by variety of environmental stresses that may be abiotic or biotic resulting in great alterations in growth and metabolism of the existing diversity (Wang *et al*., 2007; Tammam *et al*., 2011; Alqarawi *et al*., 2014). Salinity is one of the important abiotic factor having stern effects on normal growth and developmental pattern of several species (Barnawal *et al*., 2014). Increased industrialization has resulted in enhancement in the levels of salinity in most water bodies and wet lands where several species of lower plants inhabit. Estimation has been made that 7% of global landsare with high salt contents (Ruiz-Lozano *et al*., 2012) where restrictions in growth by altering the physiological and biochemical pathways are recorded. Physio-biochemical processes including photosynthesis, lipid metabolism, nitrogen assimilation and metabolism and ion homeostasis are affected adversely by salinity (Salma *et al.,* 2013; Abd_Allah *et al.,* 2015; Pade & Hagemann, 2015).

When exposed to stressful environmental conditions production and the accumulation of toxic reactive oxygen species (ROS) have been recorded by many researchers (Mittler, 2002; Wu *et al*., 2014). Increased production of ROS leads to oxidative damage and causes membrane leakage through increased lipid peroxidation (Shah *et al*., 2001). In addition, ROS has obvious effects on several other important macromolecules including proteins, nucleic acids and photosynthetic pigments (Mittler, 2002; Wu *et al*., 2014). Cells have evolved some important protective mechanisms to reduce the deleterious impact of salinity stress induced oxidative damage (Mittler, 2002). These protective mechanisms include increased synthesis and accumulation of compatible organic osmolytes, overexpression and increased antioxidants help in averting the stress induced damage (Liu *et al*., 2014; Abd_Allah *et al*., 2015). Antioxidants include both enzymatic and non-enzymatic components that mediate scavenging of these toxic ROS and thereby reducing the chances of facing oxidative stress conditions. Among the enzymatic antioxidant system are included the enzymes like superoxide dismutase (SOD), peroxidases (POD), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR). Non-enzymatic antioxidant system includes vitamins like ascorbic acid and tocopherols, glutathione and phenols etc. are among the key antioxidants involved in scavenging of toxic ROS (Mittler, 2002; Wu *et al*., 2014; Abd_Allah *et al*., 2015).

Accumulation of osmolytes like free proline, sugars and free amino acids help cells to maintain osmoregulation so that cellular water content can be maintained. Under salt stress conditions osmoregulation is achieved by the efficient uptake and accumulation of ions like sodium, chloride and potassium from the growth media. Accumulation of these organic solutes and ions with concomitant sequestration and compartmentalization of toxic ions into vacuole or least sensitive tissue spaces is an important trait determining the salt tolerance in plants (Azooz *et al*., 2011; Abd-Allah *et al*., 2015). Compartmentalizations of deleterious ions help in maintaining growth and protect various metabolic pathways (Ahmad *et al*., 2014). Toxic ROS mainly affect the membrane lipids as a result of which all biological membranes are demaged. Exposure to stress mediates conversion of unsaturated fatty acids into hydrocarbon fragments like malondialdehyde affects the permeability of

membrane (Kuznetsova *et al*., 2008; Morsy *et al*., 2012). Stress induced increase in membrane lipid peroxidation causes loss of membrane integrity and consequently affecting the nutrient efficiency (Quartacci *et al*., 2000).

Nostoc muscorum, a free living microorganism within family *Nostocaceae* inhabits both terrestrial as well as freshwater aquatic environments. *Nostoc muscorum* cells are filamentous, gram negative and green brown in color which can form spores under desiccation conditions. It is phototrophic and can also fix atmospheric nitrogen depicting its importance in nutrient cycling within the soil ecosystems. Its nitrogen fixing potential contributes in improving soil health and plant growth thriving there in. Any alterations in the optimal growth conditions can alter its normal growth. For example, Farooqui *et al*. (2011) have demonstrated reduced growth and enhanced antioxidant activity of *Nostoc muscorum* grown in higher copper concentration.

Present study was carried out to investigate the effect of high NaCl concentration on the growth and some key phsio-biochemical parameters of *Nostoc muscorum*.

Materials and Methods

Cyanobacterium, culture conditions and growth measurement: *Nostoc muscorum*, was isolated from rice field in the outskirts of Abu Hamad, Zagazig, Egypt. The identification of Cyanobacterium was carried out following the keys given by Desikachary (1959) and Starmach (1966). Axenic culture of *N. muscorum* was maintained in the culture room at 27 ± 2°C. For regular experiments, cultures were grown in nitrogen free BG-11 medium, (Stanier *et al*., 1971) at 27±2°C under photosynthetic photon flux density (PPFD) of 75 µmol m^{-2} S^{-1} and 14h photoperiod. The basal medium was supplemented with NaCl to get concentration of 0 and 250 m*M*/ L. The cyanobacteria were inoculated at the initial absorbance of 0.7 at 663 nm. To determine the growth, interval liquid culture was withdrawn from each sample and growth of cyanobacterium was recorded by measuring the absorbance of cell suspension at 663 nm using UV/Visible spectrophotometer (PG Instruments Ltd., England).

Photosynthetic pigments measurement: For determination of photosynthetic pigment, 10 ml of cyanobacterial culture was centrifuged and the pellet was suspended in acetone (80%). The cells were incubated overnight at 4°C.The suspension was centrifuged at 10,000 rpm for 5 minutes and the supernatant was used for the measurement of chlorophyll a and carotenoid contents by determining the O.D. at 665nm and 480 nm, respectively using UV/Visible spectrophotometer (PG Instruments Ltd, England). Chlorophyll a content was calculated according to Mackinney (1941). The total amount of carotenoid was calculated as per Myers & Kratz (1955).

Lipid estimation: Lipids were extracted from cyanobacterial culture pellet using a mixture of chloroform and methanol (2:1, v/v) and total lipids were estimated according to Fölch *et al*. (1957), with 0.05% (w/v) of butylated hydroxytoluene (BHT; 2.6 di-tert-butyl-p-cresol) was added to all solvents to prevent lipid peroxidation (Cachorro *et al.,* 1993). The lipid extracts were used for estimation of total lipids (Marsh & Weinstein 1966), neutral lipids (Amenta, 1964) and phospholipids (Rouser *et al*., 1970).

Lipid peroxidation (malondialdehyde content): Lipid peroxidation was determined by measuring the amount of MDA (malondialdehyde) produced by the thiobarbituric acid reaction as described by Heath & Packer (1968). The absorbance was recorded at 600 nm and 532nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM cm^{-1}.

Proline estimation: Proline was estimated following the method of Bates *et al.* (1973). Proline was extracted from cyanobacterial culture pellet (0.2 g) using 3% (w/v) sulfosalicylic acid (MP, Biomedicals, Inc.) and estimated using acid ninhydrin solution and measuring the OD at 520 nm against toluene. Standard curve of pure proline was used as a reference.

Estimation of antioxidant enzyme activities: The extraction of antioxidant enzymes was carried out as described by Malik & Singh (1980). Fresh cyanobacterial culture pellet (1.0 g) were homogenized in 50 mM sodium phosphate buffer (pH 7.0) containing 1% (w/v) PVP-40 (Polyvinylpyrrolidone) followed by centrifugation at 15,000 rpm for 20 min at 4°C. Supernatant was made to known volume with sodium phosphate buffer and was used for the enzyme assay. Proteins were estimated in the enzyme extract using method of Lowry *et al*. (1951). The activity of ascorbate peroxidase (APX) was assayed according to the method of Nakano & Asada (1981). The reaction mixture contained 1.0 ml potassium phosphate buffer (25 mM, pH 7.0), 0.1 m*M* EDTA, 0.5 m*M* ascorbate, 0.1 mM H_2O_2 and 0.1 ml of enzyme extract in a final volume of 3.0 ml. Decrease in absorbance at 290 nm was observed and activity was expressed as unit mg^{-1} protein. For the calculation of APX enzyme activity, the extinction coefficient of 2.8 mM^{-1} cm^{-1} was used. Method of Foyer & Halliwell (1976) was used to estimate the activity of glutathione reductase. In this method, the assay mixture contained 50 m*M* Na-phosphate buffer (pH 7.8), 0.12 m*M* nicotinamide adenine dinucleotide phosphate (NADPH), 0.5 m*M* oxidized glutathione (GSSG) and 0.1 ml of the enzyme extract in a total volume of 1.0 mL. NADPH oxidation was followed at 340 nm for 2 min. GR activity was expressed as l mol NADPH oxidized min^{-1} (units mg^{-1} protein). GR activity was calculated using the extinction coefficient for NADPH of 6.2 $mM^{-1}cm^{-1}$. The specific enzyme activities were expressed as EU mg^{-1} protein. Peroxidase activity was assayed spectrophotometrically according to the method of Chance & Maehly (1955). The reaction mixture contained 750 µL phosphate buffer (50 m*M*; pH 5.0), 100 µL guaiacol (20 m*M*); 100 µL H_2O_2 (40 m*M*); and 100 µL enzyme extract. OD of the reaction solution was read for 3 min at 470 nm. The enzyme activity was expressed as EU mg^{-1} protein. Catalase was estimated according to the method of Chance & Maehly (1955). The reaction mixture (2 mL) contained 1.9 mL phosphate buffer (50 m*M*; pH 7.0) and 0.1 mL H_2O_2 (5.9 m*M*) and the reaction was initiated by addition of the enzyme extract

(100 µL). Change in OD was read spectrophotometrically at 240 nm for 2 min. Enzyme activity was expressed as EU mg^{-1} protein. Superoxide dismutase (SOD) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium by adopting the method of Giannopolitis & Ries (1977). The reaction mixture consisted of 50 m*M* phosphate buffer (pH 7.8), 13 mM methionine, 60 µ*M* nitroblue tetrazolium (NBT), 0.1 m*M* EDTA, and 100 µL enzyme extract. The reaction mixture was placed 30 cm below light source consisting of two 20 W fluorescent lamps for 20 min. Another set kept in the dark served as a blank. The absorbance was measured at 540 nm. One unit of enzyme activity is the amount of enzyme required to inhibit 50% initial reduction of NBT under light.

Non enzymatic antioxidant: The level of reduced (GSH) and oxidized (GSSG) glutathione were determined fluorometrically using o–phthaldialdehyde (OPT) as fluorophore (Hissin & Hilf 1976). The level of total, reduced and oxidized ascorbate (ASC) contents in plants was measured following the protocol of Gillespie & Ainsworth (2007). In this method, plant samples (50mg) were homogenized in 1.0 ml 6% (w/v) trichloroacetic acid (TCA) under chilled conditions and centrifuged at 13,000 × *g* for 5 min at 4^0C. To 200 µL of sample 100 µL 75 mM phosphate buffer (pH 7.0) was added. In total ASC, 100 µL DTT (dithio-threitol; 10mM) was added followed by incubation for 10 min at room temperature to reduce the pool of oxidized ASC.Then,100µl NEM (N-ethylmaleimide; 0.5%) was added to remove excess DTT. For reaction, 500 µL 10 % (w/v) TCA, 400 µL 43% (v/v) orthophosphoric acid, 400 µL 4% 2, 2′-bipyridyl and 200 µL 3% (w/v) $FeCl_3$ were added to all tubes. After incubation at 37^0C for 1h, absorbance was measured at 525nm. The level of dehydroascorbate (DHA) was calculated by subtracting ASC values from total ASC. Monodehydroascorbate (MDHA) was estimated according to the method described by (Foyer *et al.,* 1989).

Results

A linear increase in growth was observed with the passage of time period from the starting of the incubation up to day eighteen (Table 1). However salinity drastically reduced the growth rate of *Nostoc muscorum* with the increase in the period of incubation. Under controlled conditions percent increase in growth on the eighteenth day of incubation was 307.2% as compared to the third day of incubation. Salinity induced percent reduction in growth rate of *Nostoc muscorum* culture was 60.33% on 18^{th} day of incubation in comparison to its respective control (Table 1).

Considerable reduction in chlorophyll contents was observed in NaCl stressed cultures. Salinity stress induced percent reduction in chlorophyll a, carotenoids and total pigments were 69.3%, 59.2% and 66.6% respectively as compared to the control (Table 2).

Results pertaining to lipid peroxidation measured in terms of MDA content and proline are depicted in Fig. 1a-b. NaCl treatment caused considerable increase in lipid peroxidation. Percent increase in MDA in NaCl treated *Nostoc muscorum* was 185.1% (Fig. 1a). Relative to control, proline accumulation enhanced in NaCl stressed *Nostoc muscorum* by 178.7% (Fig. 1b).

Results regarding the activity of antioxidant enzymes are depicted in Fig. 2a-b. NaCl stressed *Nostoc muscorum* showed increase as well as decrease in the activity of antioxidant enzymes. Relative to their respective controls, activity of CAT, POD, SOD, GR and APX increased in NaCl stressed *Nostoc muscorum* by 125.4%, 35%, 91.5%, 125.01% and 73.2% respectively whereas activity of MDHAR and DHAR reduced by 52.83% and 51.2% respectively (Fig. 2a-b).

Salinity stress caused decline in ascorbic acid whereas reduced glutathione and oxidised glutathione increased considerably in NaCl stressed *Nostoc muscorum* (Fig. 3a-c). Relative to control percent decrease in ascorbic acid content due to NaCl stress was 38.9% (Fig. 3a). NaCl increased GSH and GSSG content by 96.3% and 165% respectively (Fig. 3b).

A drastic decline was observed in the composition of major membrane lipids including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidic acid (PA) due to NaCl treatment (Table 3). Relative to control, percent reduction in phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), and phosphatidylserine (PS) was 59.5%, 32.6%, 51.4%, 96.6% and 64.2% respectively. However, phosphatidic acid (PA) content increased by 215%.

Results pertaining to effect of high NaCl on neutral lipids are shown in Table 4. Considerable variations were observed among the contents of different neutral lipids studied. Triacylglycerol (TG) and sterol (S) content was reduced by 62.6% and 11% respectively while as diacylglycerol (DG), sterol ester (SE) and non-esterified fatty acids (NEFA) increased by 31.99%, 44.30% and 49.77% respectively (Table 4).

Table 1. Influence of salinity stress (250 m*M* NaCl) on growth of *Nostoc muscorum* after different incubation periods (day).

Treatments	Incubation period (day)					
	3 days	6 days	9 days	12 days	15 days	18 days
Non salt (control)	0.234 ± 0.062	0.448 ± 0.071	0.595 ± 0.011	0.774 ± 0.028	0.884 ±.0.42	0.953 ± 0.051
Salt stress (250 mM NaCl)	0.110 ±0.003	0.255 ± 0.007	0.286 ± 0.009	0.295 ± 0.004	0.316 ± 0.009	0.378 ± 0.007
LSD at 0.05	0.072	0.104	0.107	0.115	0.185	0.124

Data presented are the means ± SE (n = 5)

Table 2. Influence of salinity stress (250 m*M* NaCl) on pigment contents (mg/g fresh weight) of *Nostoc muscorum*.

Treatments	Photosynthetic pigments (mg/g fresh weight)			
	Chlorophyll a	Carotenoids	Chl a/Carotenoids	Total pigments
Non salt (control)	4.18 ±0.125	1.528 ±0.256	2.77 ±0.011	5.709 ±0.158
Salt stress (250 mM NaCl)	1.28 ±0.027	0.622 ±0.037	2.06 ±0.034	1.906 ±0.077
LSD at 0.05	0.482	0.009	0.024	0.711

Data presented are the means ± SE (n = 5)

Table 3. Influence of salinity stress (250 m*M* NaCl) on phospholipids fractions (ug/mg algal growth) of *Nostoc muscorum*.

Treatments	Phospholipids fractions (ug/mg algal growth)					
	PC	PE	PG	PI	PS	PA
Control	1.298 ± 0.173	1.066 ± 0.052	0.772 ± 0.011	0.385 ± 0.008	0.562 ± 0.004	1.47 ± 0.091
250 mM NaCl	0.525 ± 0.082	0.718 ± 0.009	0.374 ± 0.006	0.013 ± 0.003	0.201 ± 0.055	4.64 ±0.118
LSD at 0.05	0.271	0.201	0.107	0.011	0.137	0.785

Phosphatidylcholine (**PC**); Phosphatidylethanolamine (**PE**), phosphatidylglycerol (**PG**), phosphatidylinositol (**PI**) and phosphatidylserine (**PS**), Phosphatidic acid (**PA**). Data presented are the means ± SE (n = 5)

Table 4. Influence of salinity stress (250 m*M* NaCl) on neutral lipids content (ug/mg algal growth) of *Nostoc muscorum*.

Treatments	Neutral lipids fractions (ug/mg algal growth)				
	TG	DG	S	SE	NEFA
Control	14.40 ± 2.011	8.22 ± 0.725	12.45 ± 0.803	28.17 ± 3.445	4.36 ± 0.172
250 mM NaCl	5.38 ± 0.782	10.85 ± 1.054	11.08 ± 1.007	40.65 ± 3.972	6.53 ± 0.492
LSD at 0.05	3.762	1.023	0.624	8.237	1.024

Triacylglycerol (**TG**); Diacylglycerol (**DG**); Sterol (**S**); Sterol ester (**SE**); Non-esterified fatty acids (**NEFA**). Data presented are the means ± SE (n = 5)

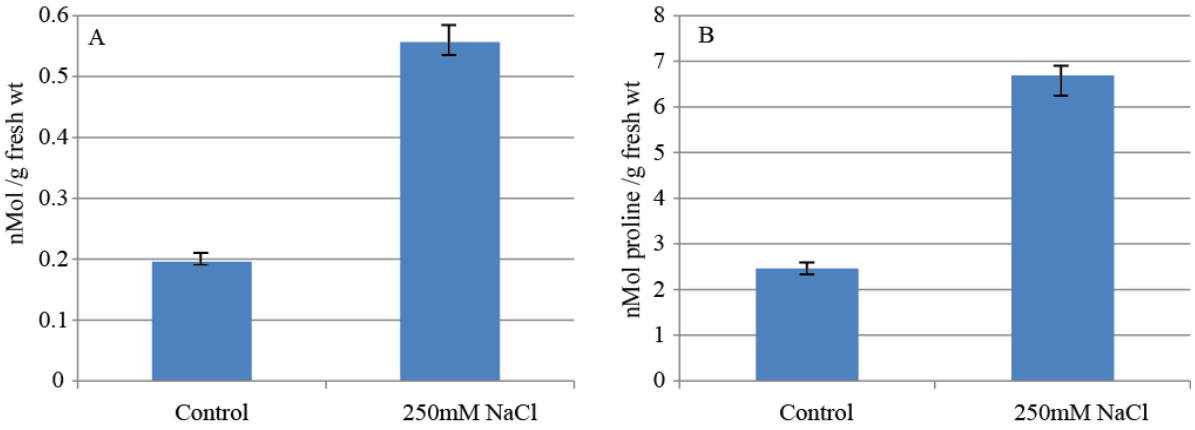

Fig. 1a-b. Effect of salinity stress (250 mM NaCl) **(A)** malonaldehyde (MDA) and **(B)** proline content in *Nostoc muscorum*. Data presented are the means ± SE (n = 5).

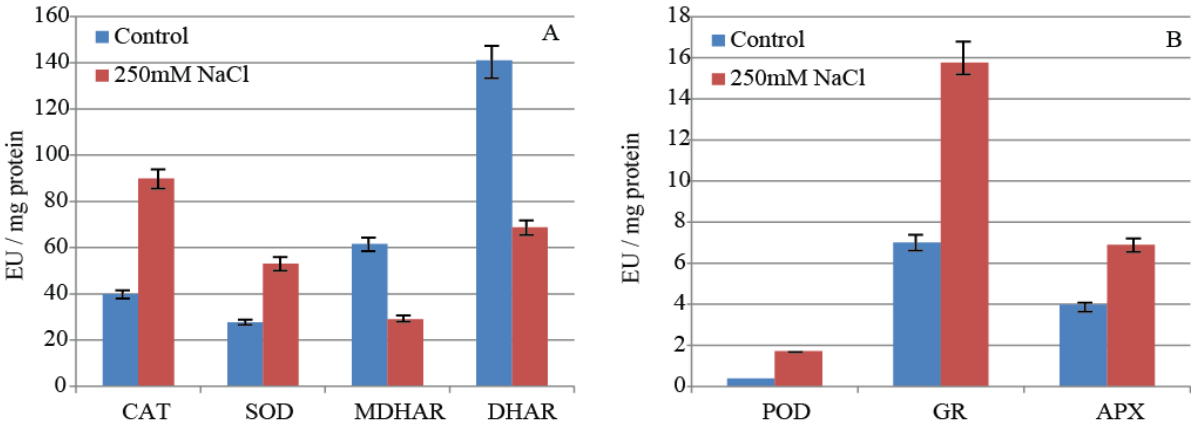

Fig. 2a-b. Effect of salinity stress (250 mM NaCl) on antioxidant enzyme activity of *Nostoc muscorum*. Data presented are the means ± SE (n = 5).

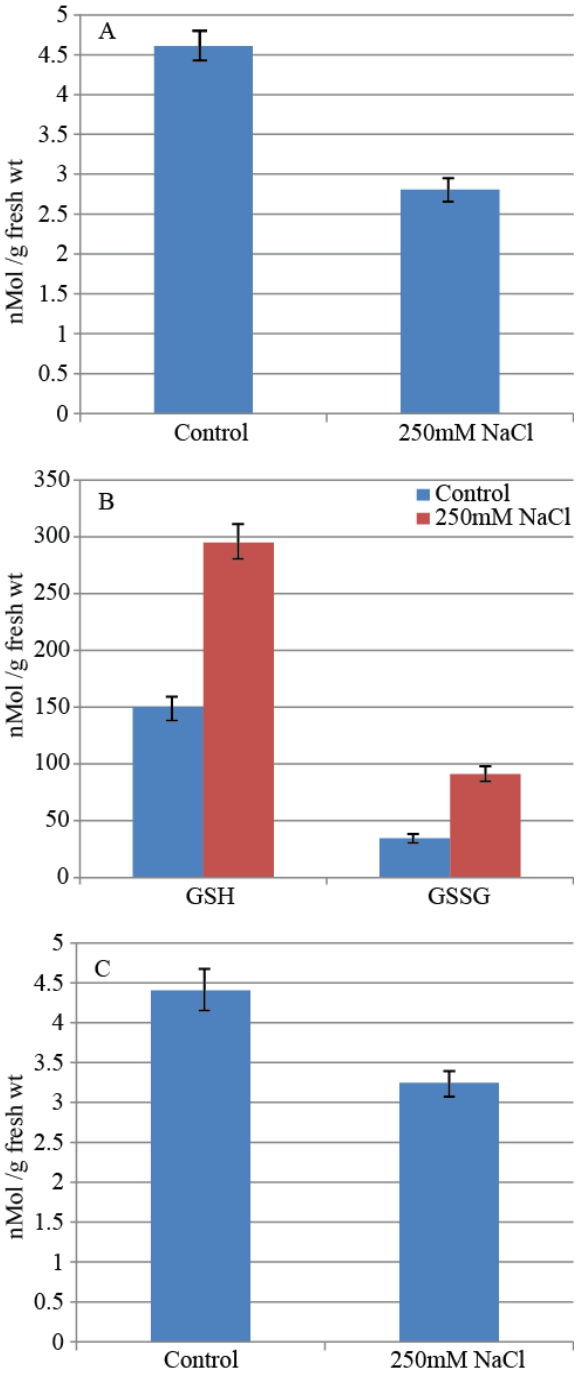

Fig. 3a-c. Effect of salinity stress (250 mM NaCl) **(A)** ascorbic acid (ASA), **(B)** reduced glutathione (GSH) and oxidized glutathione (GSSG) and **(C)** GSH/GSSSG ratio in *Nostoc muscorum*. Data presented are the means ± SE (n = 5).

Discussion

In the present study, *Nostoc muscorum* grown in cultures containing high salinity concentrations showed reduced growth and the reduction in growth was more evident with the passage of time. *Dunaliella salina* and *Dunaliella tertiolecta* (Tammam *et al.,* 2011) and Micrasterias (Darehshouri & Lutz-Meindl, 2010) grown on the media supplemented with higher salt concentrations also showed a gradual reduction in growth rate. Higher salinity induced reduction in growth is attributed to reduction in cell division and cell elongation. Our results of reduced cholorphyll pigment content in salt stressed plants are in confirmation with the findings of Wang *et al.* (2007) for *Nostoc sphaeroides* and Tammam *et al*. (2011) for *Dunaliella salina* and *Dunaliella tertiolecta*. In *Nostoc muscorum*, Srivastava (2010) has also demonstrated that increasing concentrations of NaCl in the culture media causes considerable reductions in the chlorophyll content. High salinity causes drastic reduction in the synthesis of chlorophyll pigment molecules and also causes alterations in the normal functioning of photosynthetic pigment protein complex (Levitt, 1980).

Reduced chlorophyll content in salt stressed tissues is also because of the imbalances caused in the *de novo* synthesis of proteins and other important chlorophyll components (El-Tayeb, 2005). Increased lipid peroxidation, measured in terms of malonaldehyde content, in salt stressed *Nostoc muscorum* cells support the findings of Wang *et al.* (2007) for *Nostoc sphaeroides* and Srivastava (2010) for *Nostoc muscorum*. Increased peroxidation of lipids in salt stressed *Nostoc muscorum* may be due to the enhanced synthesis of reactive oxygen species. Our results of increase in content of proline under stress conditions are corroboration with the findings of Azooz *et al.* (2011), Hashem *et al*. (2014) and Abd_Allah *et al.* (2015). In copper stressed *Nostoc muscorum*, Farooqui *et al.* (2011) have also demonstrated increased accumulation of proline resulting in mitigation of copper induced deleterious changes. Abrupt increase in proline content under salt stress is usually due to the increased activity of proline synthesizing enzymes with concomitant down regulation of proline catabolizing enzymes (Jaleel *et al.,* 2007). Increased accumulation of proline helps to maintain cellular water content well at the optimal levels so that normal metabolism may not get perturbed and the stress induced damage can be averted to some considerable extent. Proline protects protein turn over and also regulates the important stress responsive proteins (Thakur & Sharma, 2005). It has been well observed that increased accumulation of compatible osmotic solutes like proline is one of the important traits that mediate tolerance to stressful conditions (Pade & Hagemann, 2015; Abd-Allah *et al.,* 2015). Antioxidant defence system which is constituted of both enzymatic as well as non-enzymatic components help in scavenging the toxic reactive oxygen species. Antioxidant system by the ROS scavenging potential help in ameliorating the stress induced oxidative damage and its subsequent deleterious effects. By averting the stress induced oxidative stress antioxidant help in mitigating the damaging effects of toxic ROS on several important and sensitive molecules like proteins, nucleic acids and lipids. Our observations of increased activities of antioxidant enzymes like CAT, SOD, POD, GR and APX due to salt stress are in concurrence with the findings of Tammam *et al*. (2011) for *Dunaliella salina* and *Dunaliella tertiolecta* and Wang *et al.* (2007) for *Nostoc sphaeroides.* Increased activities of

antioxidant enzymes keep levels of ROS under control so that damage to important cellular components can be avoided. SOD marks first line of defense against the ROS and is involved in scavenging of superoxide radicals by dissociating them into water and hydrogen peroxide (Mittler, 2002). SOD mediated H_2O_2 produced is further converted into water and oxygen either by catalase (CAT) or ascorbate peroxidase (APX) (Mittler, 2002). In our results obvious increase in SOD, CAT and APX was observed which clearly supports the role of these key antioxidants in scavenging of ROS. Increased antioxidant activities mediate efficient and quick removal of these toxic ROS so as to keep the metabolism undisturbed as possible and keep their cellular functioning stable. In *Chlamydomonas acidophila*, Garbayo *et al.* (2007) have demonstrated that exposure to abiotic stresses (cadmium and salt stress) considerably enhanced the activities of antioxidants like glutathione reductase, ascorbate peroxidase and catalase resulting in better and quick scavenging of reactive oxygen species and hence mediating maintained growth. Monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), ascorbate peroxidase (APX) are the enzymatic components of ascorbate-glutathione cycle while as ascorbic acid and glutathione are its non-enzymatic components. Glutathione is intercoverted into its reduced form (GSH) and oxidized form (GSSG) while as ascorbic acid (ASA) is dissociated into ascorbate and dehydroascorbate. All these are the important components of ascorbate-glutathione pathway which has active role in scavenging of toxic ROS (Mittler, 2002). In ascorbate-glutathione cycle a series of well regulated and linked redox reactions are involved in which the net electron flow is from NADPH to H_2O_2 ultimately carrying conversion of H_2O_2 into water. Enhanced activity of GR increases production of reduced glutathione (GSH) and reduced glutathione is produced from the reduction of oxidized glutathione. Glutathione acts as electro donor during the conversion of dehydroascorbate (DHA) into ascorbate (ASA). ASA acts a electron donor in conversion of H_2O_2 into water and oxygen (Mittler, 2002). Higher glutathione reductase activity helps in maintaining higher ratio of $NADP^+$/NADPH so that photosynthetic electron transport and flow of electrons to molecular oxygen is maintained resulting in reduced formation of superoxide radicals (Noctor & Foyer, 1998; Mittler, 2002). Increased activity of GR also keeps GSH/GSSG ratio well maintained which is important for several physiological processes (Noctor & Foyer, 1998; Mittler, 2002). In our study reduced glutathione as well as oxidised glutathione content increased thereby keeping the electrons required for GR activity easily available. Ascorbic acid has an important role in photoprotection, regulating photosynthesis and enzyme activities containing prosthetic transition metal ions. In *Dunaleilla*, Jahnke & White (2003) observed that under elevated concentrations of salinity ascorbic acid showed a potent role in several processes. Decrease in content of ASA and increase in GSH found in our study is in concurrence with the findings of Tammam *et al.* (2011) for *Dunaliella salina* and *Dunaliella tertiolecta*. Glutathione and ASA are the important non enzymatic antioxidants protecting cells from oxidative stress through their active roles in scavenging of ROS like superoxide and hydroxyl radicals (Shan *et al.,* 2011). Reduction in phospholipids due to higher NaCl concentrations present in the media was obvious. In our results, among several phospholipids quantified almost all components e.g. phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), Phosphatidylserine (PS) showed a drastic decline with salt stress. Altered fatty acid and lipid metabolism under stressful conditions support the findings of Djebali *et al.* (2005) for *Lycopersicon esculentum* and Elloumi *et al.* (2014) for *Prunus dulcis*. In *Microcystis*, *Chlorococcum* and *Chaetoceros* Sp., Asulabh *et al.* (2012) have demonstrated that exposure to increasing NaCl concentrations caused considerable alterations in lipid profile. Lipids including Glycolipds and phospholipids are important constituents of membranes and any alteration in their content can directly alter the membrane functioning like signalling, energy transduction as well as transport (Morsy *et al*., 2012). Phospholipids estimated in our study are important constituents of membrane lipids and exposure to higher salt concentrations caused great alteration in their synthesis. This reduction in lipid content in stressed conditions may be due to the enhancement in lipoxygenase activity (Djebali *et al.,* 2005) and most sensitive are the chloroplast membrane lipids. Fatty acid in association with the galactolipids form more than 85% of total lipid component of thylakoid membranes and are important for the photosynthetic functioning of chloroplast cells (Mizusawa & Wada, 2012). Our results of increased neutral lipid content under saline conditions corroborate with the findings of Asulabh *et al*. (2012) for Microcystis, Chlorococcum and Chaetoceros Sp. Under favorable environmental conditions algae produce mainly polar lipids (glycolipids and phospholipids), thus enriching chloroplast and other cellular membranes. However, under stressful growth conditions mostly neutral lipids are accumulated in lipid droplets located in the cytoplasm (Asulabh *et al*., 2012).

Conclusion

Salinity stress reduced growth, physiological and biochemical attributes considerably. Increase in activity of antioxidant enzymes was evident in *Nostoc muscorum* contributing to the efficient scavenging of toxic ROS and thereby reducing oxidative damage. NaCl caused increase in lipid peroxidation resulting in alteration in important membrane phospholipids. However neutral lipids registered an increase with higher NaCl concentration referring to their accumulation in the lipid droplets within cytoplasm.

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