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Research Article

EFFECT OF SALINITY ON NITRATE REDUCTASE AND GLUTAMINE SYNTHETASE ENZYMES IN *CYANOBACTERIA NOSTOC LINCKIA* AND *HAPALOSIPHON SPP.*

VIVEK KUMAR YADAV*, OM PRAKASH, SINGH DV

Department of Botany, Udai Pratap (Autonomous) College, Varanasi, Uttar Pradesh, India. Email: viyadav30@gmail.com

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ABSTRACT

Effect of salinity (NaCl, 500 MM) on growth, nitrate reductase (NR), and glutamine synthase (GS) activities, and studied of the NaCl- tolerant of Cyanobacteria Nostoc linckia and Hapalosiphone spp. The past decade has seen substantial breakthroughs in understanding the biochemistry, molecular biology, and regulation of NR and GS in higher plants and blue–green algae. The objective of this study was to examine the expression and activity of NR and glutamate synthase in response to sodium chloride in halotolerant Cyanobacteria N. linckia and Hapalosiphon spp. Salinity is known to cause not only ionic and osmotic stress but also oxidative stress[1]. When a plant is exposed to high salinity, its significant procedures such as photosynthesis, protein synthesis energy, and lipid metabolisms are affected. Tijan and Ismail[2] observed that significant changes in particle and water homeostasis caused by high concentrations of salts lead to damage at the molecular level, captured growth, and even death. Environmental factors such as temperature, UV light, irradiance, drought season, and saltiness are known to influence enzymes in both Cyanobacteria and higher plants. The results of our study highlight the differential effects between the amount of sodium chloride salts on NR and GS activities in "Usar" soil Cyanobacteria N. linckia and Hapalosiphon spp.

Keywords: *Halotolerant*, *Cyanobacteria*, *Nostoc linckia*, *Hapalosiphon* spp. nitrate reductase, and glutamine synthetase.

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INTRODUCTION

Increasing soil salinity in many parts of the world has become a major problem for plant productivity [3]. The widespread distribution of *Cyanobacteria* under extreme conditions makes them a good tool to study the effect of stress conditions on cell metabolism. Salinity is an important abiotic stress for *Cyanobacteria* in aquatic and terrestrial ecosystems. In India, about seven million hectares of land area are affected by salinity and alkalinity. Such soils are collectively known as salt-affected soils and are generally reclaimed with chemical amendments such as gypsum and pyrites. The application of halotolerant strains of diazotrophic *Cyanobacteria* to such soils resulted in improved physicochemical properties of the soil [4-6]. The term salinity refers to the total concentration of dissolved inorganic ions. Thus, salinity cannot only vary in total value but also in composition. It is known that specific inorganic ions exert direct toxic effects on *Cyanobacteria*, which can restrict the occurrence of sensitive strains in certain environments. Salinity is one of the agronomist issues of worldwide concern, however, it is more serve in tropical nations [1]. Around 7 mha arrive in India is unfavorably influenced by salinity/alkalinity [7]. Salinity hinders general protein synthesis particularly stress proteins [8]. For instance, the potential for effective removal of nitrate from groundwater by *Cyanobacteria* or aquatic plants has been reported [9,10]. NaCl in trace amounts is essential for metabolic functions in *Cyanobacteria* but its elevated level inhibits growth [11]. Soil salinity is a major factor limiting agricultural productivity [12]. Growth of the crop plants is inhibited at the salinity of 50 mM NaCl and above [13], which ultimately results in yield reduction. *Nostoc linckia* is a halotolerant cyanobacterium that can grow under high salinity up to 500 mM NaCl and accumulate nitrogenous compound glycine betaine (hereafter GB) as an osmoprotectant in response to salt stress [14]. Recently, this cyanobacterium has been shown to accumulate the additional nitrogenous compound, mycosporine-2-glycine (hereafter M2G). The accumulation level of M2G also increased significantly under salt stress conditions [14]. *Cyanobacteria* are appropriate models for studying the physiology of salt tolerance and the molecular mechanism of similar processes in plants [15]. High concentration of salinity (NaCl) adversely influences the distinct active metabolic process of *Cyanobacteria* including inhibition of growth, photosynthesis, enzyme activities, and the humidity of membranes [16-18].

METHODS

Site description

For the present study, three experimental sites were identified Bunda (Site-1st), Alipur (Site-2nd) of Azamgarh district, and Bhawanipur, (Site-3rd) of Varanasi district. Site-1st (Bunda) is 106.5 km through NH 31 away from U.P. College, Site-2nd (Alipur) is 101.8 km through NH 31 away from U.P. College and Site-3rd (Bhawanipur) is 6.8 km away from U.P. College. Sites 1st and 2nd were plane by nature having kankar in scattered form and were surrounded by cultivated areas. At Site 3, some the soil surfaces were found naked and dug 3 tried dug man activities. Slopes of the whole area of the field were not equal throughout the experimental area, having stones and Kankars soil, some portion sloppy in minute stony kankars. This might be the reason for the lower biodiversity at site 3rd. The land area is unprotected and tempered by cattle.

Collection, isolation, and identification of *Cyanobacteria*

Several collections of *Cyanobacteria* were made from three different "Usar" lands/experimental sites between May 2016 and April 2018. Monthly soil sampling has been done for 2 years to see the change in soil properties and variation in *Cyanobacteria* strains. Algal patches from soil surfaces were brought to the laboratory in sterile polythene bags. The soil was dug up to a depth of 15–20 cm in length and 4 cm in diameter with the help of sterile implements and packed in polythene bags. Cyanobacterial mats were identified according to traditional methods, on the basis of morphological features and with the help of available keys [19]. The *Cyanobacteria* were then brought into the unialgal culture by transferring into liquid and solid agar media alternately. In this way, we have isolated several isolates of N2-fixing *Cyanobacteria*. Out of the available unialgal isolates, *N. linckia, Hapalosiphon* spp., *Calothrix* spp., and *Anabaena* species were chosen for further study.

Enzymatic activity

Nitrate reductase (NR)

The activity of NR in cell suspension was estimated by the calorimetric methods of Snell and Snell [20]. The theory of the method is based on the diazotization of an aromatic compound by the nitrite in solution and coupling with the reagent to give an intense red–purple color.

A known amount of induced cell suspension was taken and added to it in 100 mM KNO3 and incubated for 2 h. After that, the following reagents were added. After 15 min, the solution was centrifuged and the absorbance of the pink color was estimated at 540 nm in the calorimeter. The nitrite formed in the sample was expressed as "μ" moles NO_2 formed/μg chl a. The standard was prepared with the help of sodium nitrite.

Glutamine synthetase (GS)

The cyanobacterial suspension (10 mL) was centrifuged at 5000× g for 10 min. The pellet was washed thoroughly with 10 mL of imidazole buffer. Each pellet was suspended in 1 mL of imidazole buffer and was added 0.5 mL of toluene to each tube. The tubes were shaken gently for 3 min at room temperature and allowed to stand in an ice bucket for 20 min. Either the toluene was removed or the aliquot pipetted through the toluene 0.5 mL of cell extract was treated with 0.8 mL of the reaction mixture. The reaction was terminated after 30 min of incubation at 37°C, by adding 2.0 mL of stop mixture. The assay mixture was centrifuged at 5000× g for 10 min, and the absorbency of the coffee color supernatant was measured at 540 nm. The absorbency was converted into n mole of γ-glutamyl hydroxamate using a calibration graph prepared using 0–0.4 mL of 10 mM γ-glutamyl hydroxamate solution in the assay mixture as above. Transferase activity is expressed as n mole γ-glutamyl hydroxamate formed milligram protein min-1.

Enzyme assay

NR

None

RESULTS

A study of NR enzyme in N. linckia and Hapalosiphon spp. under salinity stress and it demonstrated vacillation for all concentrations with the exception of 500 mM. Fig. 1 portrays that NR activity was higher for 500 mM NaCl indicating an expanding design of up to 22% from the 2nd to the 8th day of inoculation, yet at the 10th day, it was diminished but hapalosiphon spp. is 23%. The rest of the concentrations such as 100, 1000, 1500, and 2000 mM demonstrated diminishing patterns in contrast with the control. On the 10th day of inoculation, NR diminished by 30% for 100 mM, 50.96% for 1000 mM, 44.60% for 1500 mM, and 34.16% for 2000 mM. Following 10 days of inoculation, the substance winds up noticeably steady for all concentrations. Analysis shows the significant result of NR and GS activities at a 0.5% level of significance.

GS activity

The amount of GS activity under salinity stress has been given in Fig. 2. GS activity for 500 mM salinity was expanded by around 10% at 25 h of

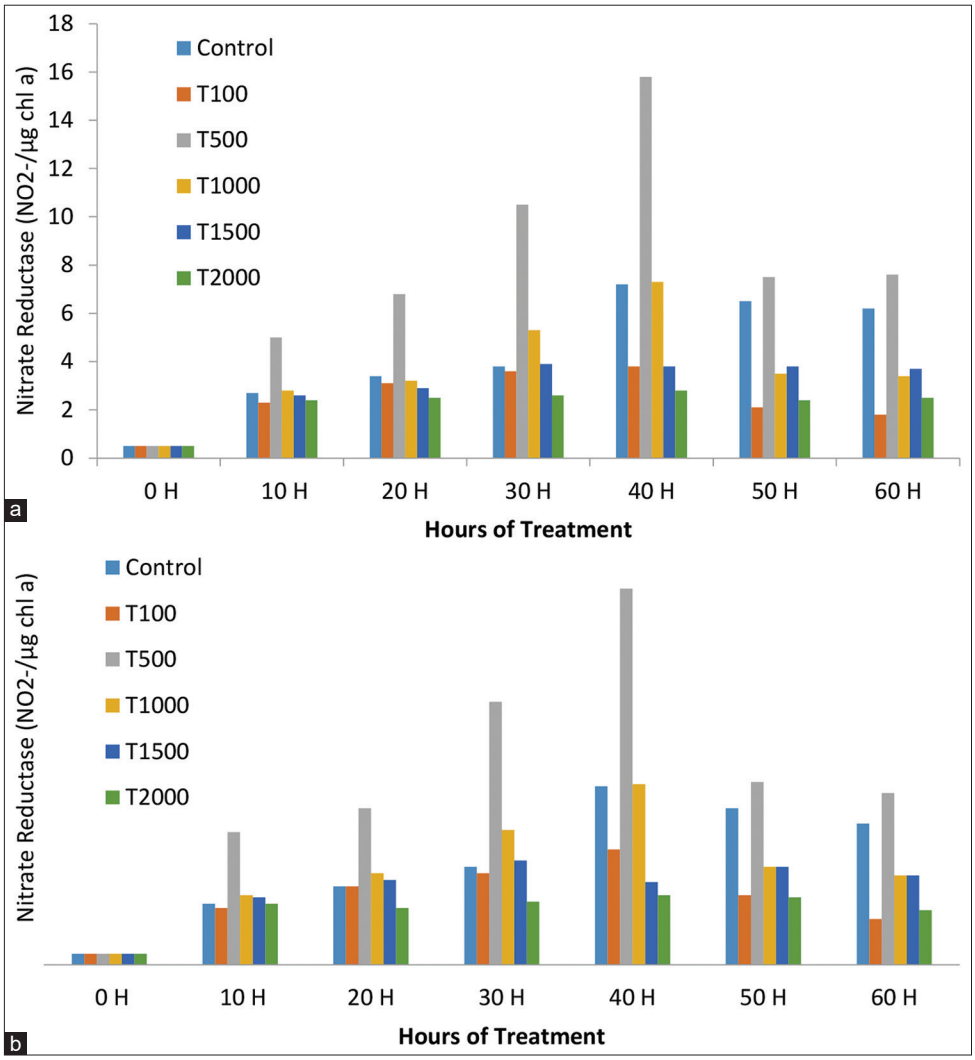

Fig. 1: Effect of NaCl on nitrate reductase activity content of halotolerant strain *Nostoc linckia* (a) and *Hapalosiphon* spp (b)

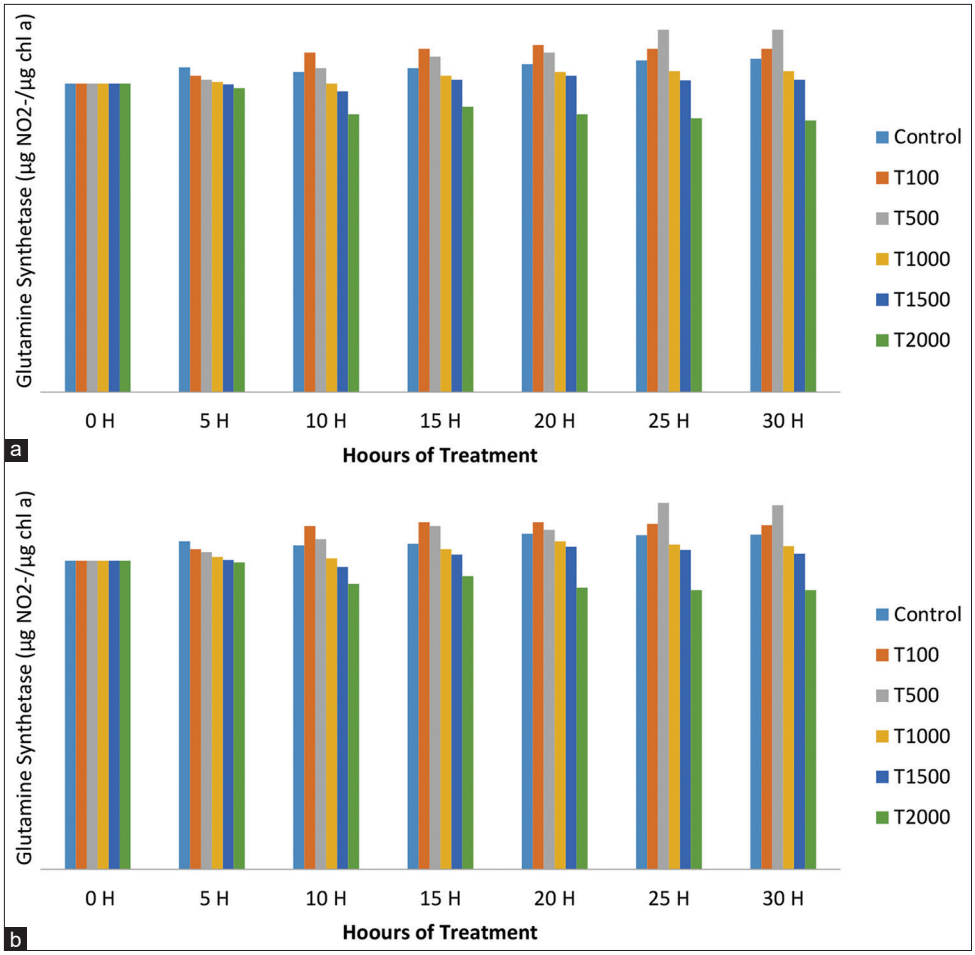

Fig. 2: Effect of NaCl on glutamine synthetase activity content of halotolerant strain *Nostoc linckia* (a) and *Hapalosiphon* spp. (b)

incubation. Non-significant increment in GS action accounted for 100 mM salinity level (nearly 3%). Up to 24 h of incubation at 1500 mM and 2000 mM of salinity, a continuous abatement of 10.21% has been found.

DISCUSSION

Nitrate is likely the richest wellspring of joined nitrogen for *Cyanobacteria* nourishment. *Cyanobacteria* especially utilize fixed nitrogen (e.g. nitrate or ammonium) and express the framework for nitrogen fixation just without a wellspring of consolidated nitrogen [21]. The specific wellspring of joined nitrogen to restrain the statement of the N_2 fixation system relies on the effectiveness with which that supplement acclimatized. Nitrate and ammonia are the best-considered nitrogen source and most likely the primary utilization nitrogen source in nature. Nitrate is the favored nitrogen source in most algal media.

The osmosis/assimilation of nitrate by *Cyanobacteria* is photosynthesis which includes the process. The producer includes (i) the passageway of nitrate into intervened by the transport system, (ii) the two-stage diminishment of nitrate is catalyzed by the ferredoxin subordinate catalysts NR and nitrite reductase (NiR) [22,23], and (iii) the joining of ammonia salts into carbon skeletons using the ATP-dependent GS and ferredoxin subordinate GOGAT system [24]. Cyanobacterial NRs are proteins of around 75 K Da constituted of a solitary polypeptide [25,26]. They contain molybdenum [26] and non-heme iron and corrosive labile sulfide [25,27], which constitutes two Fe_2S_2 bunches [27]. NR activity of *N. linckia* and *Hapalosiphon* spp. demonstrates a unique pattern than other properties, i.e., decrement seen at 100 mM grouping of NaCl. NR activity was enhanced for 500 mM of NaCl, i.e., 22.05%, yet other concentrations such as 100, 1000, 1500, and 2000 mM indicated diminishing patterns in contrast with controls and it was nearly 70.59%, 51.48%, 45.59%, and 61.77%, respectively (Fig. 1).

GS and GOGAT can consolidate settled nitrogen as ammonium into natural constituents of the cell specifically by methods of glutamate dehydrogenase (GDH) or by a cycle of responses catalyzed. The enzyme GDH catalyzes the reductive amination of two oxoglutarates to glutamate; GS catalyzes the ATP-subordinate ligation of glutamate and ammonium rendering glutamine, and GOGAT transferase the amino gathering of glutamine to 2-oxoglutarate rendering two glutamate particles. Different laborers announced that the GS/GOGAT cycle is the essential method of ammonium digestion not just in N_2-fixing *Cyanobacteria* but also additionally in non-N_2 fixing. Nonetheless, built up tentatively, by utilizing radioactive nitrogen (13N) and ammonia salts (13 NH_4^+) that GS/GOGAT cycle is the significant pathway for alkali absorption in *Cyanobacteria*.

All the make pure cyanobacterial GS proteins are defenseless to restraint by some amino acids and nucleotides [28,29] revealing aggregate hindrance of GS with a blend of aspartate, serine, and AMP, which demonstrate autonomous restricting locales for these three mixes [4] and examined the effects of different amino acids on glutamine and examined the effect of different amino acids on the glutamine synthesis movement of *Anabaena doliolum*. He found that protein is halfway responded by arginine aspartic corrosive, histidine phenylalanine, tryptophan, alanine, and glycine. Analine and glycine deliver over half restraint on glutamine synthesis movement.

CONCLUSION

The digestion of nitrate includes the movement of both NR and NiR [30]. NR is a ferredoxin-subordinate catalyst in *Cyanobacteria* [30]. In

Plasmodium tenue, NR movement in nitrate expanded with an increment in salinity. In Nostoc spp., there was no such a comparative increment in catalyst action with various salinity concentrations aside from 500 mM of NaCl. In N. calcicola, NR movement resembles the previous report [31,32]. GS is an efficient ammonia scavenger in Cyanobacteria [21]. In the present study as delineated in Figs. 1 and 2, GS action for 500 mM of salinity expanded roughly 10% at 24 h of incubation. Non-remarkable increment in GS movement accounted for at 100 mM salinity level. Up to 24 h of incubation at 1500 mM and 2000 mM of salinity, a progressive abatement of 10.21% and 19.87% has been observed. In N. linckia, the production of NR and GS, is higher than Hapalosiphon spp. In this way, it could be inferred that the impact of salinity on GS action up to 24 h was observed to be nearly insignificant.

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