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Amelioration of Salinity-Induced Metabolic Changes in *Oscillatoria willei by Gypsum*

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ABSTRACT

The present investigation was carried out to assess the effect of different concentrations of NaCl in combination with gypsum on the biochemical contents of *Oscillatoria willei* BDU 141621. In order to determine the ameliorative effect of gypsum, *Oscillatoria* was treated with different concentrations of NaCl (0.2– 0.8 M) and NaCl in combination with gypsum (10mM) besides control. The study revealed that the total chlorophyll contents increased upto 0.4M thereafter it was declined. While addition of gypsum (10mM) showed significant promotion in the total chlorophyll contents at higher levels too. The protein contents gradually decreased in all the concentrations of NaCl but gypsum treatments promoted the protein contents. While increased the accumulation of proline has been observed in all the concentrations but glycinebetaine increased up to 0.3 M thereafter declined. However addition of gypsum decreased proline and glycinebetaine contents. The study revealed that the application of gypsum could alleviate the adverse effects of salinity and increase the tolerance of alga to salinity.

Key words: ***Oscillatoria*, Gypsum, Glycinebetaine NaCl, Proline,**

INTRODUCTION

Salts are common and necessary components of soil and many salts are essential plant nutrients. But when salts are present in relatively high amounts the plant growth is adversely affected. Salinization of soil is a serious problem and is increasing gradually in many parts of the world, particularly in arid and semi arid areas. Most of the land has been reported to be saline due to indiscriminate use of chemical fertilizers, improper drainage and faulty agronomic practices. Salinity stress also leads to series of changes in basic biosynthetic functions, including photosynthesis, photorespiration and amino acid synthesis (Kawasaki *et.al.*,2001; Ozturk *et.al.*,2002; Seki *et.al.*,2002). Hence more and more areas for agricultural use are disappearing. Salinity problem is becoming more serious every year. Climate and irrigation can also influence salinity tolerance. As soil dries, salts become concentrated in the soil solution increasing salt stress. Therefore, salt problems are more severe under hot and dry conditions than under cool and humid conditions. Hence there is a pressing need to bring back such areas under cultivation and also to improve the fertility of saline soil which is utmost necessary from the agricultural point of view. Many attempts have been undertaken to counteract the adverse effects of salt stress by using chemical amendments. A widespread practice to reduce salt content in soils is leaching. With the rising cost of water, this method may not be feasible in the future. In addition, economic pressures on water supplies may force agriculture to use greater amounts of lower-quality water with higher salinity. One possible approach to reduce the effect of salinity on plant productivity is through the addition of calcium supplements to irrigation water (Sohan *et.al.*,1999; Jaleel *et.al.*,2007c).

Therefore in the present study gypsum is selected because it is an excellent and inexpensive source of calcium dissolves at high pH and it is soluble in water approximately 200 times greater than lime (CaCO3). This makes the calcium in gypsum more mobile than the calcium in lime and allows it to move more easily through the soil profile. It is reported that sulphate in gypsum is also not a problem for crops, even though it is applied in excess of plants need (Liming and warren 20110.

Recently Ca^{+2} has been reported to protect terrestrial plants against NaCl toxicity by improving the intracellular content of Ca^{+2} and maintaining Na^+/K^+ selectivity (Colmer *et.al.*,1996; Davenport *et.al.*,1997). It has been reported that Ca^{+2} concentrations and content plays an important role in the regulation of cell permeability in marine species (Krist 1977). Keeping this in view the present study was undertaken to investigate whether the addition of gypsum could ameliorate the adverse effects of NaCl stress on *Oscillatoria willei*.

MATERIALS AND METHODS

The organism used in the present study i.e., *Oscillatoria willei* BDU 141621 was obtained from National Facility for Marine Cyanobacteria (NFMC) Tiruchirapalli. ASN III medium at pH 7.5 was best suitable for the growth of the alga in the laboratory. Axenic cultures were maintained at temperature of $26^o \pm 2^oC$. Further to study the impact of NaCl and NaCl in combination with gypsum the experiments were carried out in 250 ml conical flasks, contained 100 ml of ASN III medium. In one set the cultures were treated with different concentrations of NaCl such as 0.2, 0.4, 0.6 and 0.8M besides control i.e., no NaCl and in another set different concentrations of NaCl in combination with gypsum (10mM). Similarly, the last set which contained gypsum alone and kept for observation for about 30 days. The exponentially growing algal suspension was centrifuged and inoculated in the flasks containing different concentrations of NaCl such as 0.2, 0.4, 0.6 and 0.8M and kept for observation for about 30 days.

The samples were drawn periodically during growth (10^{th}, 20^{th} and 30^{th} day) from control and different concentrations of NaCl and NaCl in combination with gypsum and gypsum alone were subjected for the analysis of biochemical parameters. The total chlorophyll contents were measured according to the method of Arnon, (1949). Total proteins were measured according to the method of Lowry *et.al.*,(1951). Proline was determined according to the method of Bates *et.al.*,(1973). Glycine-betaine was estimated by the method of Barak and Tuma (1981).All the experimental results were analysed and compared by ANOVA.

RESULTS AND DISCUSSION

Oscillatoria treated with varying concentrations (0.2-0.8M) of NaCl revealed a slight increase in the total chlorophyll contents at lower (0.2 and 0.4 M) concentrations when compared to control but it was decreased at higher concentrations (0.6 and 0.8 M) of NaCl for all the cultures studied (Table-1). According to Rai, (1990) drastic reduction in photosynthetic activity may primarily due to degradation of chlorophyll. However it has been revealed that the PS2 reaction centre is the target for salt stress in some cyanobacteria (Lu *et.al.*,1999; Lu and Vonshak 2002).While the cultures treated with gypsum showed increase in the chlorophyll contents at higher levels too, the increase in chlorophyll contents may be due to addition of gypsum. Ahmed *et.al.*,(1989) found marked growth stimulation in *Chlorella vulgaris* under certain combination of NaCl and $CaCl_2$. Calcium is known to increase halotolerance in many crop plants. Generally, Ca^{+2} had an ameliorative effect on the growth of NaCl-stressed plants, by modulating overall metabolism (Jaleel *et.al.*,2008). However it has been observed that growth inhibition induced by salt due to nutritional imbalance can be minimized with the supply of Ca along with plant nutrients (Aslam *et.al.*,1996).

Table – 1: Effect of different concentrations of NaCl and NaCl in combination with gypsum (10mM) on total chlorophyll content of *Oscillatoria willei*

NaCl/con M	**Days 10**	**20**	**30**	**NaCl+Gypsum M+mM**	**Days 10**	**20**	**30**
Control	52.0 ± 0.03	65.0 ± 0.03	81.0 ± 0.02	Gypsum only (10mM)	52.0 ± 0.01	64.0 ± 0.02	83.0 ± 0.01
0.2M	64.0 ± 0.04	79.0 ± 0.02	96.0 ± 0.03	0.1+10mM	78.0 ± 0.01	90.0 ± 0.03	102 ± 0.03
0.4M	80.0 ± 0.02	95.0 ± 0.01	114.0 ± 0.20	0.2+10mM	89.0 ± 0.02	107.0 ± 0.01	114 ± 0.01
0.6M	39.0 ± 0.01	48.0 ± 0.02	59.0 ± 0.40	0.3+10mM	109 ± 0.04	122.0 ± 0.03	132 ± 0.02
0.8M	22.0 ± 0.03	35.0 ± 0.04	30.0 ± 0.01	0.4+10mM	123 ± 0.01	138.0 ± 0.01	146 ± 0.03

Exposure to different concentrations of NaCl, *Oscillatoria* exhibited decline in the total protein contents in all the concentrations of NaCl and all the cultures studied (Table-2). Similar observations were made by Sheikh *et.al.*,(2006) in *Anabaena cylindrica* when exposed to NaCl stress. Protein degradation in a saline environment has been attributed to decreased protein synthesis, accelerated proteolysis, decreased availability of amino acids, and denaturation of enzymes involved in protein synthesis (Levitt, 1980). However Hagemann *et.al.*,(1990) reported, almost complete blockage of protein synthesis in cyanobacteria. But the gypsum treated cultures indicated increased protein content in all the concentrations of NaCl. Jaleel *et.al.*,(2008) reported that combining $CaCl_2$ with NaCl increased protein content versus NaCl stressed plants. However similar observations were made by Azooz *et.al.*,(2004) in sorghum.

Table – 2: Effect of different concentrations of NaCl and NaCl in combination with gypsum (10mM) on total protein content of *Oscillatoria willei*

NaCl/con M	**Days 10**	**20**	**30**	**NaCl+Gypsum M+mM**	**Days 10**	**20**	**30**
Control	88.0 ± 0.04	104.0 ± 0.04	121.0 ± 0.02	Gypsum only (10mM)	89.0 ± 0.03	104.0 ± 0.01	122.0 ± 0.04
0.2M	69.0 ± 0.02	84.0 ± 0.02	93.0 ± 0.03	0.1+10mM	99.0 ± 0.01	115.0 ± 0.01	132.0 ± 0.03
0.4M	52.0 ± 0.04	63.0 ± 0.04	72.0 ± 0.01	0.2+10mM	109 ± 0.03	126 ± 0.02	141.0 ± 0.01
0.6M	42.0 ± 0.03	50.0 ± 0.03	37.0 ± 0.02	0.3+10mM	128 ± 0.02	137.0 ± 0.03	156.0 ± 0.02
0.8M	22.0 ± 0.02	39.0 ± 0.02	20.0 ± 0.03	0.4+10mM	139 ± 0.01	148 ± 0.02	169.0 ± 0.01

The most distinctive change was noticed in the Proline accumulation which was increased in all the concentrations of NaCl for all the cultures studied. (Table-3). It is likely that proline accumulation may be one of the major mechanisms of salinity tolerance by the alga. It has also been reported that increase in proline contents is due to; changes in proline metabolism profile under salinity stress, increased expression of proline synthetic enzymes, breakdown of proline-rich protein (Tewari and Singh. 1991) and repressed activity of proline degradation ((Delauney and Verma, 1993; Peng *et.al.*,1996). However *Oscillatoria* treated with gypsum exhibited decline in the proline content. This is in agreement with the results obtained by Ahmed *et.al.*,(1989). This decrease may be due to the role of gypsum in modifying salt stress. Similarly NaCl-stressed plants simultaneously treated with $CaCl_2$ had lower free amino acid and proline content than plants treated with NaCl alone. Jaleel *et.al.*,(2007e) observed similar results in *Phyllanthus amarus.*

Table – 3: Effect of different concentrations of NaCl and NaCl in combination with gypsum (10mM) on proline content of *Oscillatoria willei*

NaCl/con M	**Days 10**	**20**	**30**	**NaCl+Gypsum M+mM**	**Days 10**	**20**	**30**
Control	38.0 ± 0.02	55.0 ± 0.01	73.0 ± 0.01	Gypsum only (10mM)	39.0 ± 0.01	56.0 ± 0.02	74.0.0 ± 0.01
0.2 M	63.0 ± 0.04	88.0 ± 0.02	108.0 ± 0.03	0.1+10mM	54.0 ± 0.03	63.0 ± 0.03	79.0 ± 0.03
0.4 M	87.0 ± 0.01	110.0 ± 0.01	127.0 ± 0.04	0.2+10mM	45.0.0 ± 0.02	52.0 ± 0.01	64.0 ± 0.02
0.6 M	107.0 ± 0.03	128.0 ± 0.02	140.0 ± 0.02	0.3+10mM	31.0 ± 0.03	43.0 ± 0.04	52.0 ± 0.01
0.8 M	121.0 ± 0.01	142.0 ± 0.01	168.0 ± 0.03	0.4+10mM	24.0 ± 0.01	30.0 ± 0.02	42.0 ± 0.03

It is interesting to note that glycinebetaine content also increased with increase in NaCl concentrations upto 0.6 M and thereafter declined (Table 4). Glycinebetaine is the principal solute in highly salt tolerant halophilic forms. Several studies reported that, glycinebetaine is characteristically found in most of the cyanobacteria under inhospitable conditions including *Aphanotheca halophytica* and *Synechocystis* DUN 52 (Reed *et.al.,*1984). Similar observations were made by Gabbay-Azaria *et.al.,*(1988) in *Spirulina subsala.* It has been reported that glycinebetaine preserves thylokoid and plasma membrane integrity when exposed to saline solutions (Rhoades and Hanson, 1993). It has also been revealed that glycinebetaine stabilizes the structure and activity of enzymes and maintains the integrity of membranes against the damaging effects of excessive salt, cold, heat, and freezing (Gorham, 1995). However gypsum treatment reduced glycinebetaine content by modifying salt stress. The gypsum alone did not show much change in any of the cultures studied when compared to control.

Table – 4: Effect of different concentrations of NaCl and NaCl in combination with gypsum (10mM) on glycinebetaine content of *Oscillatoria willei*

NaCl/con M	**Days**			**NaCl+Gypsum M+mM**	**Days**		
	10	**20**	**30**		**10**	**20**	**30**
Control	81.0 ± 0.02	95.0 ± 0.02	105.0 ± 0.01	Gypsum only (10mM)	82.0 ± 0.03	96.0 ± 0.01	106.0 ± 0.04
0.2 M	120.0 ± 0.01	131.0 ± 0.03	145.0 ± 0.02	0.1+10mM	97.0 ± 0.03	102.0 ± 0.01	110 ± 0.01
0.4 M	143.0 ± 0.03	154.0 ± 0.02	170.0 ± 0.01	0.2+10mM	75.0 ± 0.02	83.0 ± 0.02	95.0 ± 0.02
0.6 M	157.0 ± 0.02	172.0 ± 0.01	181.0 ± 0.03	0.3+10mM	63.0 ± 0.01	71.0 ± 0.01	81.0 ± 0.01
0.8 M	108.0 ± 0.01	120.0 ± 0.01	131.0 ± 0.01	0.4+10mM	51.0 ± 0.02	60.0 ± 0.02	69.0 ± 0.03

CONCLUSION

The study indicated that, *Oscillatloria willei* exhibited various response to different concentrations of NaCl. However the application of gypsum exhibited enhancement in the chlorophyll and protein contents in all the concentrations of NaCl. While proline and glycinebetaine were reduced by the application of gypsum. In conclusion, these beneficial properties indicated that the application of gypsum could ameliorate salinity-induced metabolic changes by modulating overall metabolism of *Oscillatoria willei*

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