J. Algal Biomass Utln. 2010, **1** (3): 43 – 53 Alleviation of Saline Stress by Gypsum in *Chlorella sp*

JOURNAL OF ALGAL BIOMASS UTILIZATION

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Alleviation of Saline Stress by Gypsum in *Chlorella vulgaris* BEIJERINCK

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ABSTRACT

In the present study the interactive effect of NaCl and gypsum ($CaSO_4.2H_2O$.) on biochemical contents of *Chlorella vulgaris Beijerinck* was investigated. In order to evaluate the role of gypsum in the alleviation of saline stress, *Chlorella* was treated with different concentrations (0.1-0.4M) of NaCl and NaCl fortified with gypsum (10mM) besides control. The study revealed that the total chlorophyll contents increased upto 0.2M 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 their contents. While NaCl treatments increased the accumulation of proline 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 gypsum treatment could alleviate the adverse effect of salinity on *Chlorella vulgaris.*

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

INTRODUCTION:

Salinity is one of the major obstacles for increasing production of crops throughout the world. It has become an ever-increasing problem in irrigated areas. However salinity is also considered as one of the major constraints on species diversity and productivity of algae (Booth and Beardall 1991; Chen and Plant 1999). There are several studies on the effect of NaCl on microorganisms. Many attempts have been undertaken to counteract the adverse effects of salt stress by using chemical amendments. However gypsum is the most commonly used agricultural amendment for the alleviation of saline stress. Muhammad, (1998) reported that, use of gypsum ($CaSO_4.2H_2O$) as a source of Ca^{2+} is a well established practice for the amelioration and management of Na^+ saturated water/ soils. Calcium is an important factor in the resistance of plants to salinity. Abdel-Basset (1993) found that,Ca^{2+} in certain ratios to Na^+ reversed most of NaCl stress symptoms in *Chlorella vulgaris*.However, Lilley (1971) made an observation that, salt tolerance can be increased by the addition of Ca salts in higher plants. In the present study, an attempt has been made to investigate whether supplementary gypsum can alleviate the adverse effects of saline stress on *Chlorella vulgaris* Beijerinck.

MATERIALS AND METHODS

The organism used in the present study i.e., *Chlorella vulgaris* Beijerinck was isolated from the university garden soil. De’s modified Beneck’s medium was found best suited for the growth of the alga in the laboratory. The cultures were maintained in the culture room at temperature of 26 ± 2°C.

Further to study the impact of NaCl and role of gypsum in alleviation of saline stress the experiments were carried out in 250ml conical flasks, contained 100ml of Bold's basal medium. In one set the cultures were treated with different concentrations of NaCl such as 0.1, 0.2, 0.3 and 0.4M besides control i.e., no NaCl and in another set different concentrations of NaCl fortified with gypsum (10mM).Similarly, the last set which contained gypsum alone 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 fortified with gypsum and gypsum alone and were subjected for the analysis of biochemical parameters. The total chlorophyll contents were measured according to the method of Arnon (1949), total proteins by the method of Lowry *et al*. (1951), proline was determined according to the method of Bates *et al.* (1973) and Glycinebetaine was estimated by the method of Barak and Tuma (1981). All the experimental results were analyzed and compared by Anova using SPSS Package version 10.

RESULTS AND DISCUSSION

The results indicated that the total chlorophyll contents increased at lower concentrations (0.1 and 0.2M) of NaCl when compared to control but it was decreased at higher (0.3 and 0.4M) concentrations for all the cultures studied (Table1).According to Moradi and Ismail (2007) reduced chlorophyll contents at higher salinities are due to decrease in photosynthetic rate because of salt osmotic and toxic ionic stress.

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. Shaddad *et al*., (1989) observed enhanced pigment biosynthesis when higher plants exogenously supplied with vitamins. However Aslam *et al*., (1996) made an observation that growth inhibition because of salt induced nutritional imbalance can be minimized with the supply of Ca along with plant nutrients.

Table-1: Effct of NaCl ,gypsum (10mM) and NaCl plus gypsum on total Chlorophyll and total proteins contents of *Chlorella vulgaris Beijerinck.*

	Total chlorophyll (μg/gFW)			**Total proteins(μg/g FW)**		
Con. of NaCI	10th day	20th day	30th day	10th day	20th day	30th day
Control	46.0 ± 0.03	55.0 ± 0.03	72.0 ± 0.02	73.0 ± 0.04	90.0 ±0.02	104.0 ±0.02
0.1M	58.0 ± 0.04	67.0 ± 0.02	89.0 ± 0.03	58.0 ± 0.02	70.0 ±0.04	81.0 ± 0.03
0.2M	72.0 ± 0.02	88.0 ± 0.01	101.0±0.03	41.0 ± 0.02	52.0 ±0.02	61.0 ± 0.01
0.3M	31.0 ± 0.02	42.0 ± 0.02	56.0 ± 0.04	30.0 ± 0.04	38.0 ±0.01	29.0 ± 0.02
0.4M	19.0 ± 0.02	28.0 ± 0.04	30.0 ± 0.01	12.0 ± 0.02	20.0 ±0.02	18.0 ± 0.03
Gypsum only	48.0±0.02	58.0±0.01	75.0±0.01	76.0±0.02	92.0±0.01	07±0.03

10mM						
0.1M+10mM	76.0±0.01	95.0±0.03	113±0.03	98.0±0.01	112.0±0.01	120.0±0.03
0.2M+10mM	93.0±0.02	118±0.01	128±0.01	114.0±0.03	126.0±0.02	132.0±0.01
0.3M+10mM	115.0±0.04	130±0.03	143±0.02	129.0±0.02	139.0±0.03	146.0±0.02
0.4M+10mM	132.0±0.01	147±0.01	158±0.03	141.0±0.01	152.0±0.02	162.0±0.01

Each value is expressed as mean ± S. D. (n=3) X Statistically significant at P < 0.05.

FW=Fresh Weight

Table-2: Effect of NaCl, gypsum (10mM)and NaCl plus gypsum on proline and glycinebetaine contents of *Chlorella vulgaris Beijerinck*.

	Proline(µg/gFW)			**Glycinebetaine(µg/gFW)**		
Con. Of NaCl	**10th day**	**20th day**	**30th day**	**10th day**	**20th day**	**30th day**
Control	30.0 ± 0.02	46.0 ± 0.01	62.0 ± 0.02	69.0 ± 0.02	78.0 ±0.01	99.0 ± 0.01
0.1 M	58.0 ± 0.04	69.0 ± 0.01	96.0 ± 0.04	98.0 ± 0.01	110.0 ± 0.02	130.0 ± 0.02
0.2 M	79.0 ± 0.01	98.0 ± 0.02	120.0 ± 0.02	122.0 ± 0.02	135.0 ± 0.01	146.0 ± 0.03
0.3 M	98.0 ± 0.02	119.0 ± 0.03	138.0 ± 0.03	141.0 ± 0.03	149.0 ± 0.02	151.0 ± 0.01

0.4 M	115.0 ± 0.01	131.0 ± 0.02	150.0 ± 0.03	102.0 ± 0.02	110.0 ± 0.03	121.0 ± 0.02
Gypsum only (10mM)	31.0±0.02	48.0±0.02	64.0±0.03	70.0±0.01	80.0±0.02	99.0±0.01
0.1M+10mM	33.0±0.03	40.0±0.03	49.0±0.03	59.0±0.03	61.0±0.01	71.0±0.01
0.2M+10mM	27.0±0.02	31.0±0.01	38.0±0.02	42.0±0.02	50.0±0.02	60.0±0.02
0.3M+10mM	20.0±0.03	22.0±0.04	27.0±0.01	30.0±0.01	41.0±0.01	51.0±0.01
0.4M+10mM	12.0±0.01	15.0±0.02	19.0±0.03	21.0±0.02	25.0±0.02	31.0±0.03

Each value is expressed as mean ± S. D. (n=3) X Statistically significant at $P < 0.05$.

FW=Fresh Weight

Chlorella exhibited decline in the total protein contents in all the concentrations of NaCl up to 30th day (Table 1). The present results are in agreement with the results of Rahman *et al*. (2005). Hageman *et al*. (1990) found complete blockage of protein synthesis in cyanobacteria. But the gypsum treated cultures indicated increased protein content in all the concentrations. Similarly many previous studies reported that application of thiamin (B_1) enhanced protein accumulation in *Chlorella vulgaris* and *Ankistrodesmus falcatus* (Makled, 1995).It is interesting to note that proline content increased in all the concentrations of NaCl (Table 2). The accumulation of proline may be one of the major mechanisms of salinity tolerance by the alga. According to the observations of Imamul-Hug and Larther (1985), accumulation of proline may be considered as an indication of a salt induced water deficit. *Chlorella* 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. Glycinebetaine is the principle solute in highly salt tolerant halophilic forms. It was also increased with increase in NaCl concentrations up to 0.3M and thereafter declined (Table 2). Record *et al.* (1988) reported that, osmolytes like glycinebetaine and proline increase the cytoplasmic volume and free water content and permit cell proliferation under unfavorable conditions. Several species of marine algae have been reported to contain glycinebetaine as a stabilizing osmolyte. Reed *et al.* (1984) reported that, glycinebetaine is characteristically found in most of the cyanobacteria under inhospitable conditions .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.

CONCLUSION :

The results of the present study indicated that application of gypsum increased the chlorophyll and protein contents in all the concentrations of NaCl. However proline and glycinebetaine were reduced by the application of gypsum. In conclusion, the application of gypsum could alleviate the adverse effects of salinity and increase tolerance of alga to salinity by modifying saline stress with reduced proline and glycinebetaine contents.

Acknowledgements: The authors are thankful to the Chairman, Department of Botany, Gulbarga University, Gulbarga for providing the necessary facilities.

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