INFLUENCE OF CYANOBACTERIAL FILTRATE ON GROWTH OF RICE SEEDLINGS UNDER SALINE CONDITIONS

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ABSTRACT

In the present investigation, effect of extracellular polymeric substances (EPS) *i.e.* extracellular polysaccharides and proteins, produced by *Anabaena sphaerica* (a halotolerant heterocystous cyanobacterium), on the growth of rice seedlings was studied under varying salinity regimes of EC 0.2, 5, 10 and 15 dS/m. A positive correlation was found between the salinity and EPS produced by cyanobacterium. Amendment of cyanobacterial filtrate resulted in increased growth of seedlings showing 9 to 210% increase in radicle growth and 8 to 65% increase in plumule growth under saline conditions. These results indicate that salt stress get alleviated and salt induced inhibition of growth get diminished due to the presence of EPS in the filtrate.

Key words: Salinity, *Anabaena sphaerica*, EPS, Rice seedlings.

I. INTRODUCTION

Soil salinity is one of the major abiotic stresses which adversely affects the growth of crop plants (Shahbaz and Ashraf, 2013). Nearly one billion hactares (about 7%) of the total land area of world is salt affected (Yensen, 2008) & in India, about 7 million hectares of land is salt affected (Patel et al, 2011) and mostly in the states of Haryana, Punjab, Uttar Pradesh, Gujrat & Rajasthan (Shrivastava and Kumar, 2015) etc. Rice, one of the staple food is most susceptible to salinity than the other cereals and young seedlings or the early developmental stages are the most sensitive to salt stress (Hussain and Jin, 2017; Soltani et al., 2006). Cyanobacteria (= blue green algae) play an important role as biofertilizer in fields due to their nitrogen fixing capability (Singh et al, 2014). There are reports of amelioration of saline soils by the application of cyanobacteria ((Rai, 2005; Aziz and Hashem, 2003). Application of cyanobacteria results in improvement of the soil structure, decrease in electrical conductivity (Nisha et al, 2017) & pH (Prabhu and Udaysooriyan, 2007). Cyanobacterial exudates like extracellular polysaccharides, having binding property, improve soil characteristics and stimulate growth of crop seedlings ((Nisha et al., 2007)). The present work is aimed to analyze the bioameliorative characteristics of an indigenous halotolerant strain of cyanobacterium *Anabaena sphaerica*, with an objective to explore its potential as a biofertilizer and the effect of its exudates on the growth of rice seedlings under different salinity levels.

II. MATERIALS AND METHODS

Anabaena sphaerica isolated from saline alkali soils of Rohtak, Haryana was cultured in Fogg's nitrogen free medium (Fogg, 1949) maintained at 27±3ºC under continued light intensity of 2000 lux (Kaushik, 1987). Halotolerance potential of *A. sphaerica* was studied at different salinity levels of EC 0.2, EC 5, EC 10 and EC 15dS/m using heteroionic solutions having NaCl, Na_2SO_4, $MgCl_2$ & $CaSO_4$ in a ratio of 13:7:1:4 by weight (Sinha et al., 1986) which is general soil composition in this region. The earlier studies have showed that *A. sphaerica* is a halotolerant alga, able to grow at higher salinity levels of EC 15 dS/m although with reduced biomass, chlorophyll, carotenoids and phycobilins (Manchanda, 2015).

Bioameliorative Characteristics were analyzed in the exponential growth phase on 21st day.

Extracellular polysaccharides (EPS): For quantitative estimation of EPS, 100 ml of each of the actively growing culture of *Anabaena sphaerica* maintained at four different salinity levels of EC 0.2, 5, 10 and 15 dS/m were taken and centrifuged at 5000 rpm for 10 minutes. Supernatant obtained was concentrated to half of its initial volume by boiling. Supernatant was cooled and 15 ml of 95% ethanol was added to precipitate out the EPS. Precipitates were washed with ethanol and weighed after drying, first at room temperature & then overnight in a dessicator over conc. H_2SO_4 (Moore and Tischer, 1965).

Extracellular Protein: Extracellular proteins were estimated by following Lowry et al. (1951). To 1ml of algal filtrate, 5 ml of alkaline biuret reagent was added, mixed well & allowed to stand at room temperature for 20 minutes. 0.5 ml of folin - ciocalteau reagent was added and after 30 minutes absorbance was taken at 700 nm. The amount of protein was estimated from the standard curve prepared from graded conc. of bovine serum albumin.

For estimation of **sodium uptake** by the alga, 200 mg of algal pellet obtained by centrifugation of 15 day old culture was digested with 1ml of 60% per-chloric acid, 5 ml conc. nitric acid and 1 ml conc. sulphuric acid at 70^{o}C for 4 hours and volume was raised to 100 ml using glass distilled water. Concentration of sodium in the extract gave the total sodium removed by the cyanobacterium (Allen et al, 1986).

Effect of cyanobacterial filtrate on rice seedlings

21 day old culture filtrate of cyanobacterium was taken and centrifuged at 10,000 r.p.m for 15 minutes. The collected supernatant was concentrated to 10% of its original volume in rotary vacuum evaporator at 35^{o}C (Misra and Kaushik, 1989) and the concentrated supernatant was used for amendement.

Rice seeds (IR-64) were used to study the effect of filtrate of *Anabaena sphaerica* on the growth of rice seedling. For this healthy seeds were selected, washed with distilled water & surface sterilized using 70% ethanol for 30 seconds followed by 5 minutes treatments with $HgCl_2$. Seeds were washed repeatedly with distilled H_2O. Four sets of petridishes (one for each salinity treatment) in triplicates were used, which were padded with sterilized moist filter paper. 30 seeds were arranged in each petridish & were incubated at 30^{o}C in BOD incubator in dark. After three days 5 ml of concentrated supernatant was added to germinated seedlings and incubated further for five days & length of radicle and plumule of seedlings was measured.

III. RESULTS AND DISCUSSION

Production of extracellular polysaccharides and protein by *Anabaena sphaerica* significantly ($p<0.01$) increased with increasing salinity. *Anabaena sphaerica* produced high amount of extracellular polysaccharides (3 to 6 times) under saline conditions as compared to control indicating that high concentration of salts promotes the production of exopolysaccharides (EPS). Similarly, extracellular proteins also increased with increasing salinity. An increase in extracellular polymeric substances with increased NaCl concentration had also been reported by earlier workers (Sheng et al, 2006). Table 1 shows that with increased level of extracellular polymeric substances (EPS), sodium absorbed and adsorbed by the alga is more under saline conditions (25.5 µg/ml, 165.8 µg & 168.1 µg/ml at EC 5, 10 & 15 dS/m) than the control (16.9 µg/ml). This can be due to the chelation of sodium ions by the chemical ligands present in EPS (Aksu et al., 2002). Removal of sodium by alga helps in lowering the salinity of the medium and if this alga is introduced in the saline soils, it would be helpful in lowering the electrical conductivity as reported in earlier studies by El-Sheekh et al. (2018).

Table 1: Effect of salt stress on Bioameliorative characteristics of *Anabaena sphaerica*

Salt Conc. (ds/m)	Extracelllular polysaccharides (mg/ml)	Extracellular proteins (mg/ml)	Sodium Adsorbed (mg/ml)	Sodium Absorbed (mg/ml)	Biomass (mg/ml)	Sodium uptake (µg/ml biomass)
EC 0.2	0.29	69	0.008	0.011	0.65	16.9
EC 5	0.81	78	0.028	0.013	0.51	25.5
EC 10	1.17	56	0.025	0.063	0.38	165.8
EC 15	1.73	40	0.017	0.054	0.32	168.1

When three days old germlings of variety IR-64 were treated with concentrated algal filtrate of *Anabaena sphaerica* it was found that radicle and plumule length increased. In control (EC 0.2 dS/m) medium, radicle and plumule length increased after treatment with *Anabaena sphaerica* filtrate. Results in Table 2 indicate that there is a decrease of 16 to 78% in radicle length and 19 to 70% decreased in plumule length in the unamended rice germlings with increasing salinity. This decrease in radicle and plumule growth may be due to synthesis of metabolic inhibitors by seedlings or due to inhibition of utilization of carbohydrates and nitrogenous substances under saline conditions (Strogonov, 1962). By the application of algal filtrate, suppressing effect of salinity was reduced to 4 to 40% and 12 to 55% in case of radicle & plumule length. These results also show that there is nearly 9 to 210% and 9 to 170% increase in radicle length and 2 to 42 % and 8 to 65 % increase in plumule length after 3 days and 5 days of amendment. This increase in growth of seedlings in terms of radicle and plumule growth due to amendment of *Anabaena sphaerica* filtrate was found to be statistically significant ($p<0.05$) under saline conditions. This study shows that most likely these extracellular products act as source of energy (carbon and nitrogen) for the germinating rice seedlings and thus promoting their growth.

Table 2. Effect of application of *Anabaena sphaerica* filtrate on the radicle and plumule growth of rice germlings at varying salinity levels.

Treatment	Days after treatment	Radicle length (cms)				Plumule length (cms)			
		EC 0.2	EC 5	EC 10	EC 15	EC 0.2	EC 5	EC10	EC15
Unamended	0	1.82	0.87	0.43	0.45	1.14	0.97	0.48	0.21
	3	6.06[a]	5.1[a]	4.75	1.36[a]	4.87	3.94	2.03[a]	1.44
	5	8.54	5.4[x]	5.04	2.36[x]	4.93[x]	5.34	3.26[x]	2.55[x]
21 day Old Filtrate	3	7.57[a]	7.27[a]	5.19	4.19[a]	4.55	4.0	2.87[a]	2.04
	5	7.9	7.76[x]	5.46	6.26[x]	6.51[x]	5.76	4.44[x]	4.31[x]

Values with same superscripts under each column are statistically different from one another as shown by t- test ($p < 0.05$)

These results are in harmony with the earlier studies by Herrero et al. (2001) & El-Nahas et al. (1999). Similar kind of results were obtained in earlier studies by Rodriguez et. al. (2006) which showed that NaCl (5gm/l) resulted in reduction of shoot length (54%) and root length (62%) in *Oryza sativa* & EPS of cyanobacterial filtrate results in reduced suppression of growth by reverting the effect of salinity. Arora et al. (2010) had also shown that application of cyanobacterial exudates promote growth as sodium ions, which otherwise have inhibitory effect on seeed germination and seedling growth, get bound to EPS and are no more available as free ions in the medium and thus alleviate the adverse effect of salinity. Further, stimulation in seedling growth may be attributed to presence of growth promoting hormones like auxins and gibberellins in EPS of cyanobacterial filtrate (Essa et al., 2015).

IV. CONCLUSION

This study shows that application of filtrate of *Anabaena sphaerica* promotes growth of seedlings significantly under saline conditions. This is due to the presence of extracellular polysaccharides and proteins which help in binding the Na^+ ions and hence, alleviation of the salt stress. Thus, on the basis of these results, further studies may be conducted to find out the potentiality of this strain of cyanobacterium to be used for bioamelioration of saline soils & for improving seed germination and seedling growth under salt stress.

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