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Impact of Graded Concentration of KCl on the Growth and Heterocyst Frequency of Parent and Mutant Strain of *Anabaena variabilis* RDU-1

Mridul Singh[1*], Jai Prakash Pandey[2] and U. K. Chauhan[1]

[1]Department of Biotechnology, A.P.S. University, Rewa(M.P.), [2]Department of Biotechnology, Govt. T.R.S. College, Rewa(M.P.). *mksingh.biotech@gmail.com

ABSTRACT

Cyanobacteria are oxygenic, photosynthetic prokayotes growing and multiplying in a wide range of ecological habitats ranging from hot springs to Antarctic. In the present study, the parent and multiple herbicide resistent mutents of *Anabaena variabilis* , RDU-1 strains were taken for examining their relative response to salt stress under diazotrophic growth conditions. Mutant strains differentiated heterocyst with more or less similar frequency in all the concentration of KCl ranging from 20 mM to 100 mM in comparision to parent strain decreased even in 20 mM to KCl. Heterocyst differentiation is salt tolerant in the mutant strain and salt sensitive in parent strain.

Keywords: *Anabaena variabilis*, Heterocyst, Mutant strain and Parent strain.

INTRODUCTION

Cyanobacteria as biofertilizer for rice cultivation have a beneficial effect on crop productivity and maintenance of soil fertility. The diazotrophic Cyanobacteria includes non-heterocystous, unicellular forms, filamentous and heterocystous forms. Heterocystous cyanobacteria are the most efficient aerobic N_2 – fixers under oxygenic photosynthetic conditions than nonheterocystous forms mainly because of heterocyst.[1] Heterocystous nitrogen fixing cyanobacteria are the natural components and water logged rice field and tropical countries like India where they grow luxuriantly and fix nitrogen abundantly.[2]

In this present investigation, the parent and multiple herbicide resistant mutant of *Anabaena variabilis*, RDU-1 strains were taken for examining their relative response to salt stress under diaxotrophic growth conditions.

MATERIALS AND METHODS

Source of Organism, culture medium and cultivation

The cyanobacterium *Anabaena variabilis*, RDU-1 its multiple herbicide resistant mutant used in the present investigation was obtained resistant mutant used in the present investigation was obtained throug Department of Biological Sciences, R. D. University, Jabalpur. The organism is a local isolate from, rice field of Jabalpur. The multiple herbicide resistant mutant of *Anabaena variabilis*, RDU- 1 isolated through spontaneous mutation showed resistance to four commonly used rice field herbicides Arozin, Alachlor, Butachlor and 2. 4-D. The organism differentiates three kind of cells i.e. vegetative cells, heterocysts and akinetes under nitrogen fixing condition.

In order to obtain clonal population of the cyanobacterium, the filaments were homogenized by vigorously using glass beads in a vortex mixer. 2-4 called trichomes thus produced were washed five times with sterile medium by centrifugation and streaked on agar plates. The plates were incubated under photoautotrophic growth conditions in a culture room maintained at 25± 1^0C. Algal colonies developed after 10 days of incubation were picked up and transferred to a liquid medium. The axenic status of the culture was tested from time to time by streaking cells on nutrient agar media. Bacteria free colonies were picked up with sterile glass capillaries under binocular microscope and incubated aseptically into sterile culture tubes containing 5 ml fresh N_2 medium.

BG-II medium without combined nitrogen source was used as basal medium (designated as N_2 medium) for routine cultivation of *Anabaena variabilis*. The multiple herbicide resistant mutant strain was maintained with growth, toxic level of the herbicides. The culture medium, glassware and chemicals were sterilized by autoclaving at pressure of 15 lb/inch^2 (121^0C) for 15 minutes.[3]

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Growth Conditions

The clonal culture of parent and mutant of *Anabaena variabilis* RDU-I was maintained in bacteria free state by routinely transferring (at interval of 6-7 days) aseptically, the mid exponential phase culture to 200 ml fresh medium contained in 500 ml conical flasks. The cultures were grown photoautotrophically in a culture room maintained at (25±2^{0}C) and illuminated with cool florescent light at photon flux density of 2500 lux at the surface of the vessel for 16 hours a day.

Heterocyst Frequency

It was determined by counting the number of heterocyst in a given filament and expressed as % frequency

Impact of graded concentration KCl on the growth and heterocyst frequency of parent and mutant strain of *Anabaena variables* RDU-1

Effect of increasing concentrations of KCl of (0, 20, 40,60,80 and 100mM on the growth and heterocyst frequency of multiple herbicide Alachlor, Butachlor, Arozin, 2-4-D, resistant strain of *Anabaena variabilis*, RDU-1 was determined in N2 medium by monitoring in the concentration of heterocyst frequency at regular intervals for 12 days.

Mutant strains differentiated heterocyst with more or less similar frequency in all the concentration of KCl raging from 20 mM to 100mM in comparison heterocyst differentiation and frequency of parent strain decreased even in 20mM to 100mM of KCl. Microscopic observation reveal that salt concentration of 40mM and above induced fragmentation of filaments is parent culture, this effect of salt was comparatively much less in mutant strain. Thus to conclude heterocyst differentiation is salt tolerant in the mutant strain and salt sensitive in parent strain. This type of studies has also done by Singh et. al., 2007.[6]

RESULTS AND DISCUSSION

Heterocystous form or photosynthetic aerobic N_2 fixers with photosynthetic carbon assimilation taking place within vegetative cell and nitrogen fixation taking place in heterocyst. Heterocyst is the known site of the nitrogenase activity because of its role in activation of Nif gene expression and in protection of O_2 toxicity. The known biochemistry and morphology associated with heterocyst differentiation from vegetative cell are the facts in support of the observed conclusion when a vegetative cell differentiates into a heterocyst fixing nitrogen.[4]

KCl is a necessary ingredient in the growth medium of diazotrophic and non-diazotrophic cyanobacterium both. Potassium is required as a physiological nutrient for maintenance of enzyme stability and activity as well as maintenance of cell osmolarity.[5]

The result suggests that ammonium nitrogen is an inhibitor for heterocyst differentiation and that heterocyst differentiation is a requirement for the diazotrophic growth. Both the strains were also compared for heterocyst frequency as shown in (Table - 1). Thus the mutant strain differ from parent strain were also compared in respect of their response to heterocyst differentiation and frequency to varying concentration of KCl.

Table 1 - Impact of graded concentration of KCl on heterocyst frequency (%) of parent and multiple herbicide Al^r, But^r, Aro^r, and 2-4-D resistant mutant of *Anabaena variabilis* RDU-1.

Days	Control		20mM		40mM		60mM		80mM		100mM	
	P	M	P	M	P	M	P	M	P	M	P	M
0	6.8	4.3	6.8	4.3	6.8	4.3	6.8	4.3	6.8	4.3	6.8	4.3
2	7.5	4.6	7.4	4.5	7.2	4.4	6	6.8	4.3	6.6	4.3	6.3
4	8.1	4.7	7.9	4.6	7.6	4.5	7.2	4.5	6.9	4.4	6.7	4.4

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6	8.9	5.0	8.2	4.8	8.0	4.7	7.8	4.7	7.2	4.5	7.0	4.5
8	9.2	5.3	8.6	4.9	8.2	4.9	8.0	4.9	7.5	4.7	7.1	4.7
10	9.6	5.6	8.9	5.2	8.6	5.2	8.2	5.2	7.8	4.8	7.3	4.8
12	9.8	5.8	9.0	5.4	8.7	5.4	8.4	5.4	7.8	5.0	7.4	4.7

CONCLUSION

This paper has demonstrated that heterocyst differentiation is salt tolerant in the mutant strain and salt sensitive in parent strain. The resulting knowledge for the given study would provide basis to introduce salinity tolerant phenotype into the multiple herbicide resistant cyanobacterial strains for its ultimate use as an appropriate biofertilizer strains with potential for application in rice agriculture.

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