Table 1 *Influence of soil fertility on the level of soluble protein in mustard irrigated with saline waters*

Salinity levels (meq l)	Soluble protein (mg/g dry wt)					
	45 DAS			65 DAS		
	IF	LF	Mean	IF	LF	Mean
0	285.8	236 8	261.3	217.7	200.5	209.1
50	280 8	221.5	251.2	208.9	186.3	197.6
100	236 9	211.5	224.2	189 8	176.5	183.2
150	250.8	203.5	227.2	193.5	163.3	178.4
Mean	263.6	218.3		202.5	181.7	
LSD at 5%						
Salinity (S)	7.34		4 95			
Fertility (F)	5.19		3.50			
S × F	10.39		7.00			

The merit of this interaction in terms of improvement of plant performance and yield will be reported subsequently.

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SHORT-TERM EFFECT OF UREA ON NITROGENASE ACTIVITY IN *NOSTOC ANTHOCEROS*

SURENDRA SINGH and R. PRAKASHAM
Department of Biochemistry, North-Eastern Hill University, Shillong 793 014, India.

NITROGENASE is found exclusively among prokaryotic organisms. All nitrogenases isolated so far are very similar in their molecular structure[1]. Nitrogenase activity in heterocystous cyanobacteria is located in heterocysts which are the sites of nitrogen-fixation[2]. Nitrogen-fixation is an energy expensive process and therefore, the enzymatic activity of nitrogenase and its biosynthesis is subjected to regulation by ammonia and other nitrogen sources[3,4]. *Nostoc anthoceros* which has stimulated considerable interest owing to their ability to fix N in association with the liverwort *Anthoceros punctatus*. Regulation of cyanobacterial nitrogenase in relation to ammonia metabolism has been reported[5,6], but to date very little data are available on the effect of urea on nitrogenase activity[7]. In this paper we describe the short-term effects of urea on nitrogenase activity of *N. anthoceros*. For this purpose the uptake of urea and the nitrogenase activity have been studied in *N. anthoceros* cultures exposed to urea, methionine sulphoximine (MSX) and adenosine triphosphate disodium salt (ATP).

Axenic batch cultures of *N. anthoceros* were grown in BG-11 medium[8] under continuous light at 50 μmol photons.$m^{-2}.s^{-1}$ at 24 ± 1°C. Exponentially growing culture (7 days) were used for each experiment and the urea concentration was 1 mM. MSX (250 μM) and ATP (50 μM) were added to the cultures 2 h prior to the addition of urea and incubated under normal growth conditions so as to allow the entry of the chemical into the cells. Urea uptake was estimated by measuring its depletion from the external medium as described by Rai and Singh[9]. Nitrogenase activity was measured following the method of acetylene reduction assay[10]. Protein was estimated by the method of Lowry *et al*[11] standardized with bovine serum albumin. Chlorophyll was estimated using the method of Mackinney[12].

Table 1 shows changes in nitrogenase activity levels and urea uptake rates in *N. anthoceros* grown in N-free medium and then transferred to urea-containing medium. Cells grown under N_2-fixing conditions were able to assimilate urea, accompanied by a decrease in nitrogenase activity. Urea decrease in nitrogenase occurred only after a lag period of 1 h. This period was consistent with the necessity for urea to be first hydrolysed to ammonia which may be the active repressor in this case. After 2 and 5 h, cells exhibited an appreciable urea uptake rate (540.50 and 523.87 nmol/mg protein/h, respectively) and nitrogenase activity was about 55 and 10%, respectively of the original value. Our observation also supports the proposition[9] that during short-

Table 1 *Urea, MSX and ATP-induced changes in nitrogenase activity and urea uptake levels in diazotrophically grown Nostoc anthoceros*

Incubation period (h)	Urea uptake rate (nmol/mg protein/h)			Nitrogenase activity (nmol C_2H_4.μg Chl/*a*/h)		
	Urea	Urea + MSX	Urea + ATP	Urea	Urea + MSX	Urea + ATP
0	0.0	0.0	0.0	3.63	3.63	3.63
1	525.95	520.69	550.56	3.49	3.57	3.60
2	545.50	542.35	562.50	2.02	3.52	2.98
3	550.45	552.47	575.75	1.20	3.33	2.87
4	549.33	550.00	625.00	0.76	3.15	2.67
5	523.87	519.23	605.31	0.37	2.97	2.59

All data are the mean of four independent determinations.

term experiments (up to 1 h) urea uptake is free from its subsequent hydrolysis in NH_3 and CO_2. It could also be seen that ammonia inhibition of nitrogenase is rather similar to that produced by urea (data not shown). These results strongly suggest that products from ammonia assimilation are responsible for urea inhibition of nitrogenase activity. To test whether urea promoted nitrogenase inhibition was due to the metabolism of the internally generated ammonium, experiments were carried out in which glutamine synthetase (GS), the first enzyme involved in ammonia assimilation had been inactivated by 2 h of prior incubation with MSX. MSX prevented nitrogenase activity inhibition by urea without impairing the ability of the cells to take up urea. These results indicate that MSX-treated cells escape nitrogenase activity regulation by urea because the production of ammonium derivatives is interrupted. Since, the same treatment also abolished the ammonium-induced nitrogenase switch-off (data not shown), the present data reinforce our proposal that nitrogenase activity regulation by urea is mediated by products from its assimilation rather than by urea itself.

The incorporation of ammonia through glutamine synthetase/glutamate synthase (GS/GOGAT) is an ATP-dependent processes[13]. The cells receiving ATP exogenously demonstrated a pattern of urea uptake similar to that observed with control cells (urea only) while an increased level of nitrogenase activity was observed as compared to ATP-untreated cells. This indicates that ammonia produced by urea hydrolysis inhibits nitrogenase activity by altering the ATP needed for nitrogenase activity. Our observation also supports earlier findings that assimilation of ammonia by GS/GOGAT pathway limits the availability of ATP for nitrogenase[14].

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