EFFECT OF CERTAIN CHEMICALS ON THE *IN VIVO* STATUS OF THE ENDOSYMBIONT *ANABAENA AZOLLAE* IN *AZOLLA PINNATA* R. BR.

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THE nitrogen-fixing cyanobacterium *Anabaena azollae* is an endosymbiont in the aquatic plant *Azolla pinnata*. Biological nitrogen fixation by the *Azolla–Anabaena* complex is considered to have potential to increase rice yield at comparatively low cost[1]. There are a few earlier reports on the effect of growth regulators such as indoleacetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), gibberellic acid (GA) and MH as well as the effect of salt stress on the growth of *Azolla*[2–5], but their impact on the endosymbiont is not known. The present investigation was undertaken to determine the effect of these substances on the *in vivo* status of the endosymbiont under nitrogen-fixing conditions.

Azolla pinnata plants grown in a nitrogen-free medium[6] were treated with IAA, 2,4-D, naphthaleneacetic acid (NAA), GA, kinetin, ABA and colchicine at concentrations of 1, 2, 5 and 10 ppm, and KCl and NaCl at 1, 10, 50 and 100 mM. They were incubated at 27 ± 2°C under fluorescent tubes (2500 lux) in a 14/10 light/dark cycle. After 15 days the cyanobacteria were isolated from the basal cavities of the fifth leaf from the tip and the total number of vegetative cells as well as heterocysts were determined.

The population of *Anabaena* cells was about 4000 and the heterocyst frequency was 17% (table 1). There had been no proper estimation of cyanobacterial population in the leaves, although there are many reports on the heterocyst frequency[7–12]. The earlier reports on heterocyst frequency give figures between 15 and 45%. It has also been reported that the heterocyst frequency increases from near zero at the shoot apex to 30–45% in the 15th leaf[11,12]. Therefore, based on our standardization, the fifth leaf was chosen for the experiment. IAA, ABA and NaCl at all the concentrations used and KCl at 10 and 50 mM did not significantly influence cyanobacterial population and heterocyst frequency (table 1). Although ABA is a potent growth-inhibiting hormone of higher plants[13], it did not adversely affect the endosymbiont or *Azolla*[6]. The synthetic auxins 2,4-D and NAA exhibited adverse effects even at very low

Table 1 *Effect of plant growth regulators on cyanobacterial population and heterocyst frequency*

	Number of cells*				Heterocyst frequency (%)			
Chemical	1	2	3	4	1	2	3	4
IAA	3971	3540	3959	3650	18	18	18	18
2,4-D	1656	1420	—	—	17	10	—	—
NAA	1940	1525	—	—	16	15	—	—
GA	4141	4601	4092	4650	18	18	18	18
Kinetin	4417	4211	1791	1350	18	18	10	5
ABA	3445	3796	3850	3850	17	17	17	17
Colchicine	4950	4511	4500	4350	18	18	18	18
KCl**	4015	3816	3850	620	18	16	16	9
NaCl**	3950	3500	3615	3500	17	17	17	17
Control		3945				17		

*Each number is mean of three values.
Concentrations: 1, 1 ppm; 2, 2 ppm; 3, 5 ppm; 4, 10 ppm; **1, 1 mM; 2, 10 mM; 3, 50 mM; 4, 100 mM.

concentrations (1 and 2 ppm) and were lethal at higher concentrations. A similar adverse effect was also observed with higher concentrations of kinetin and KCl, although they had a favourable effect on cyanobacterial population at low concentrations. GA was the only phytohormone that caused increase in cyanobacterial population and heterocyst frequency at all the concentrations tested. Colchicine, a potent alkaloid capable of inducing polyploidy[14], was used to study its effect on *Azolla*, and surprisingly it caused an increase in cyanobacterial population as well as heterocyst frequency at all the concentrations tested.

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EFFECT OF CYCLOHEXIMIDE ON METABOLISM OF PYRIMIDINES IN COTYLEDONS OF GERMINATING BLACK GRAM SEEDLINGS

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MARKED changes in the patterns of metabolism of pyrimidines have been observed in germinating seeds of leguminous plants[1–3], but detailed mechanisms of such changes remain to be resolved. The relative activities of the enzymes already present in dry seeds and those synthesized during germination seem to play a major role. Thus, we have compared the metabolism of pyrimidines in black gram cotyledons incubated with or without cycloheximide, an inhibitor of protein synthesis in eukaryotic cells.

Seeds of black gram (*Vigna mungo* L.) were sterilized and germinated on 0.55% agar-gel with or without cycloheximide (25 $\mu g.ml^{-1}$) in the dark at 27°C under aseptic conditions[3]. More than 90% of the incorporation of [^{3}H]leucine into the trichloroacetic acid-insoluble fraction was inhibited by this concentration of cycloheximide[4]. After 24 or 48 h, each pair of cotyledons was divided into 6 segments. Eighteen segments from three pairs of cotyledons were incubated in 2 ml of sodium phosphate buffer (10 mM, pH 6) that contained 0.3% glucose and labelled compounds (37 KBq) for 4 h at 37°C. The specific activities of [2-^{14}C]uracil, [2-^{14}C]uridine and [6-^{14}C]orotic acid were 2.00, 1.97 and 2.18 MBq.μmol^{-1}, respectively. Labelled compounds were analysed by the methods described earlier[3]. The activities of uracil phosphoribosyltransferase, uridine kinase, orotate phosphoribosyltransferase (OPRTase) plus orotidine-5'-monophosphate decarboxylase (ODCase) and phosphoribosylpyrophosphate (PRPP) synthetase in cell extracts were determined as described earlier[5].

Table 1 provides details of metabolism of [2-^{14}C]uracil, [2-^{14}C]uridine and [6-^{14}C]orotic acid in cotyledons incubated with and without cycloheximide. In day-old cotyledons, approximately 45% of exogenously supplied [2-^{14}C]uracil was incorporated into nucleotides and nucleic acids. More than half of the [2-^{14}C]uracil was catabolized reductively, most of the radioactivity being released as $^{14}CO_2$, with some found in β-ureidopropionate, an intermediate in the reductive degradation of pyrimidines[6]. The anabolic metabolism of uracil decreased in two-day-old cotyledons, while the catabolic metabolism of uracil increased. In both day-old and two-day-old

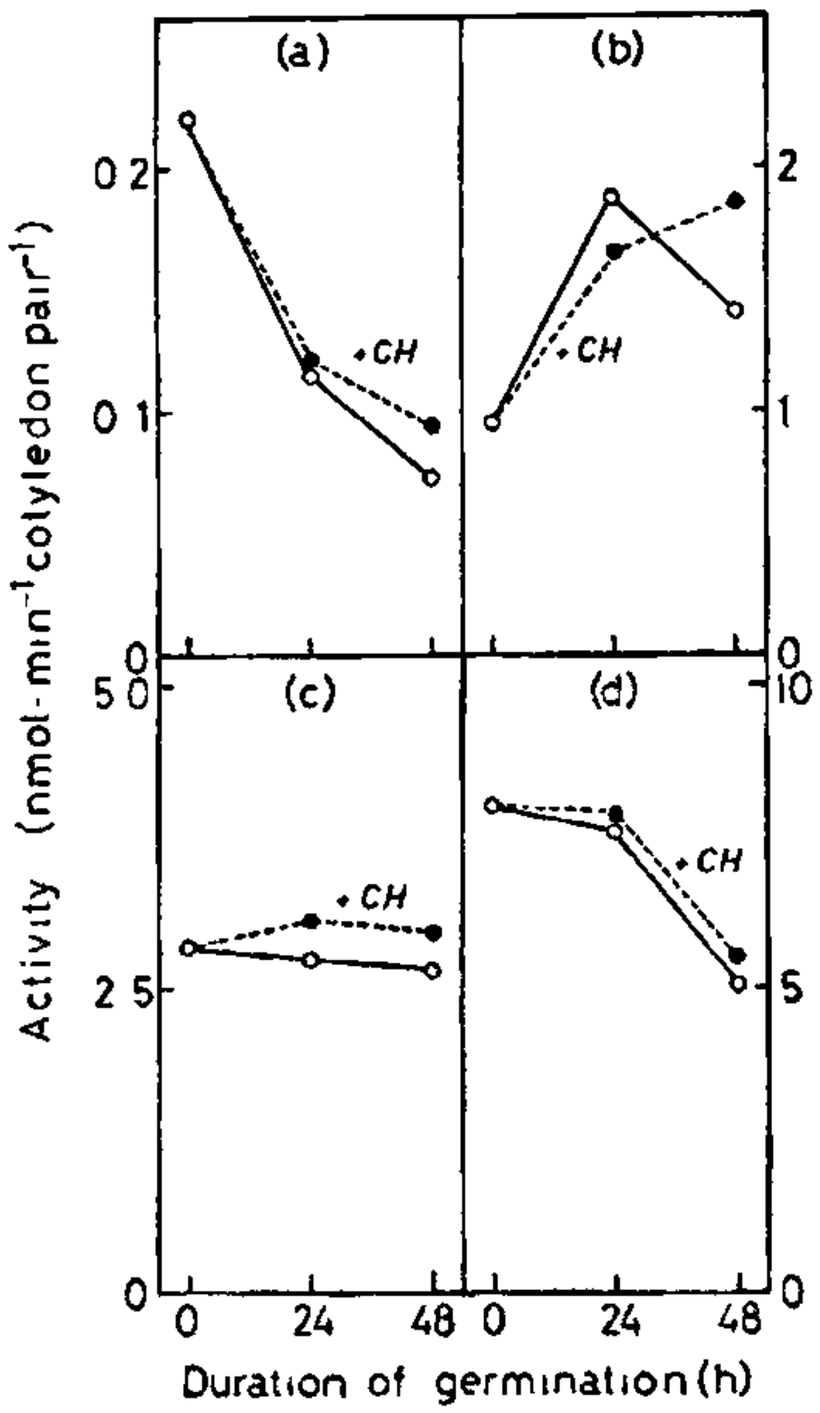

Figure 1a–d. a. Changes in activities of uracil phosphoribosyltransferase; b. Uridine kinase; c. Orotate phosphoribosyltransferase and orotidine-5'-monophosphate decarboxylase; d. Phosphoribosylpyrophosphate transferase in cotyledons of black gram seedlings incubated with (-●-) or without (-○-) cycloheximide (25 $\mu g.ml^{-1}$). The enzymatic activities are expressed as nmol per min per pair of cotyledons. The values are averages of results from duplicate samples from a typical experiment.