To date, the molecular mechanisms of colchicine stimulation of growth have not been studied. The observations of KING[16] seem to indicate that they could be similar to the gibberellin-induced α-amylase biosynthesis. This author has shown that colchicine (1.0 μM) stimulates the liberation of sugars in the wheat seed during germination. In addition, the demonstration that colchicine binds in vitro to DNA molecules[17] points out again that its site of action may be at the gene level. Besides, it is plausible to foresee that colchicine produces its effect of growth stimulation in a way similar to the induction of c-mitosis. That is to say, due to its capacity to bind to subunit protein of microtubules of the mitotic apparatus[18].

In brief, the mechanisms underlying the stimulatory effect of colchicine remain unclear. Nevertheless, the afore-discussed would seem to indicate that colchicine – at low concentration – may well be effective as a promoter of cell division, and thereby prepare the tissues for its ulterior mutagenic activity at an higher concentration. It may be concluded therefore, that the cellular mechanism of colchicine action has a twofold function: the stimulation of the nuclear division and of cytokinesis, followed by the induction of polyploidy. One practical consequence from these findings might be the use of colchicine in a dosage sequence for increasing the percentage of polyploid cells in the tissues.

Effect of colchicine on the germination of wheat seeds

Colchicine concentration (μM)	Duration of treatment (h)	
	48	56
0	58	69
0.1	65	72
10.0	69	86
1000.0	76	93

Samples of 30 seeds were incubated in the dark at 24 °C temperature in an aqueous solution of colchicine and in distilled water. The results are given as percentage of seeds germinated at the end of the incubation periods.

Résumé. Le développement de la racine et de la coléoptile est stimulé lorsque des plantules de Graminées sont incubées avec la colchicine à des concentrations au-dessous du seuil qui produit ses effets mutagéniques. Cette stimulation de la croissance – qui semble être due à une activité méristématique accrue – met en évidence un double aspect du méchanisme d'action de la colchicine: la stimulation de la prolifération cellulaire, suivie de l'induction de la polyploïdie. En outre, ceci suggère que le traitement des organes avec des doses de colchicine chaque fois plus élevées devrait permettre d'augmenter le nombre de cellules polyploïdes dans les tissus.

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Fixation of Elementary Nitrogen by *Nostoc punctiforme* (Kütz.) Hariot and *Scytonema bohneri* Schmidle in Pure and Unialgal Cultures

A number of blue-green algae have been shown to fix elementary nitrogen in pure cultures. Association of other micro-organisms, especially bacteria, with these algae have been shown to result in increased nitrogen fixation, and in most of the cases the total nitrogen fixed was higher than that fixed by either of the organisms when grown singly[1-3].

During a survey of the soil algae from the rice fields, a large number of blue-green algae have been isolated in unialgal cultures. Many of these are found to thrive well on a culture solution free from nitrogen. In the present investigation the nitrogen fixation capacity of 2 algae has been studied and compared in both unialgal and pure cultures. Unialgal cultures of *Nostoc punctiforme* (Kütz.) Hariot and *Scytonema bohneri* Schmidle, isolated from rice field soils of Ballia and Ghazipur (U.P., India), respectively, were raised in pure bacteria-free cultures by use of UV-irradiation coupled with streaking on silica gel plates after the methods of GERLOFF et al.[4], and WATANABE[5]. Purity of cultures was tested with peptone solution (1%), mixture of glucose (0.5%) and peptone (1%) solutions, meat extract ('Oxo-Lablemco') solution (1%) and DE's[6] liquid solution containing glucose (0.5%).

The cultures were grown at 28–30 °C under continuous fluorescent light (180 Lux) in 250 ml Pyrex Erlenmeyer flasks, containing 100 ml of DE's[6] nitrogen-free medium, supplemented by modified HUTNER's[7] micronutrient solution, in which $(NH_4)_6Mo_7O_{24}$ was replaced by MoO_3 in order to make it nitrogen-free as described earlier[3]. Initial pH of the solution was adjusted to 7.0.

The cultures were harvested after 35 days of growth and the purity of the cultures was retested. The algal mat of each replicate was separated by centrifugation. Nitrogen was estimated both in the supernatant and the algal mat

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using conventional microkjeldahl method[8]. The dry weight of the algal mat was taken only of the pure cultures by filtration through pre-weighed Whatman filter paper (No. 42) and drying in an oven. Dry weight of unialgal cultures were not taken as the alga was growing with other bacterial contamination and the dry weight could not have been assigned to either of the organisms. The results obtained are shown in the Table.

It is evident from the results presented in the Table that the pure cultures of *N. punctiforme* and *S. bohneri* are capable of fixing elementary nitrogen. They also confirm our earlier work with *Scytonema hofmanni* Ag., ex. Born et Flah., and *Fischerella muscicola* (Thuret) Gomont[3], that the total nitrogen fixed in unialgal cultures is higher than the nitrogen fixed in pure cultures.

In the present investigation with *Nostoc punctiforme*, the total nitrogen fixed in unialgal culture is about 5 times more than the nitrogen fixed in pure culture of the alga. Slight increase is also noticed in unialgal culture of *Scytonema bohneri* over the amount of nitrogen fixed in pure culture. BUNT[1] obtained about 200% increase in total nitrogen fixed by an unidentified species of *Nostoc* when grown with *Caulobacter*, a bacterium which was prominent among the contaminants. He further found that the *Caulobacter* was incapable of growing and fixing elementary nitrogen alone in pure culture in a nitrogen-free medium, even when supplied with a suitable carbon source. BJÄLFVE[2] showed nitrogen fixation in pure cultures of *Nostoc calcicola* and a substantially larger fixation was obtained when *Nostoc* was combined with other micro-organisms (bacteria or actinomycetes). Maximum nitrogen fixation was observed (5.42 mg) when *Bacillus megaterium* was grown in association with *Nostoc calcicola* and this was 500% over that of pure culture of *Nostoc calcicola*, a value very close to that observed in our experiment with *Nostoc punctiforme* where an increase of more than 400% was observed in unialgal culture over the pure culture of the alga.

Nitrogen fixation by *Nostoc punctiforme* has also been reported by others and as tabulated by PANKOW and MARTENS[9], DREWES[10] found 0.07–0.12 mg and WINTER[11] 0.054–0.394 mg nitrogen per 10 ml culture solution with algae in 60 and 75 days respectively in a culture medium without molybdenum. BORTELS[12] found 0.69–0.81 mg and 0.38–0.63 mg nitrogen in 31 days per 10 ml culture solution in presence and absence of molybdenum respectively. Calculated this way, the nitrogen fixation by *Nostoc punctiforme* in our experiment would be 0.0916 mg in 35 days per 10 ml culture solution containing molybdenum.

PANKOW and MARTENS[9] considered *Nostoc sphaericum* to be the best fixer among the known *Nostoc* species, as they showed fixation of 0.615 mg nitrogen in 90 days and 1.05 mg nitrogen in 140 days per 10 ml culture solution. Calculated per dry weight it comes to 5.16% and that of *Nostoc punctiforme* in our experiments is 12.05%. STEWART[13] showed nitrogen fixation in pure cultures of *Nostoc entophytum* to be 5.83% of dry weight. It is difficult to compare our results with the various other reports made on *Nostoc* since they lack dry-weight data.

Reports about the nitrogen fixation in the genus *Scytonema* is scanty. FULLER et al.[14] reported nitrogen fixation in *Scytonema hofmanni* B and F and *Scytonema archangelli*, and the percentage of nitrogen fixed was 5.7% and 9.9% per dry weight of the alga respectively. They did not, however, mention the purity of algae from bacteria other than azotobacter, volume of basal nutrient solution used for growing the algae and incubation period. The percentage of nitrogen fixed per dry weight, as reported by us earlier[3], with pure cultures of *Scytonema hofmanni* was 3.89 and in the present investigation with *Scytonema bohneri* is 4.98, a value quite close to that obtained by FULLER et al.[14].

Not much is known about the symbiotic relationship of microflora present in the soil. Results presented here and those reported earlier[1–3] are indicative of enhanced fixation when algae grow in association with other micro-organisms. However, it remains to be discovered whether the associated organisms contribute nitrogen independently or promote in some way the fixation capacity of the algae, as has been shown by BUNT[1] and BJÄLFVE[2,15].

Zusammenfassung. Während Stickstoffixierung durch viele Blaualgen bekannt ist, wird diese Fähigkeit von *Scytonema bohneri* hier zum erstenmal gemeldet. Bei *Nostoc punctiforme* erreicht die N-Fixierung höhere Werte als bisher von irgendeiner Blaualge bekannt war und ausserdem zeigt sich das bereits bekannte Phänomen, dass kontaminierende Bakterien den Ertrag an gebundenem Stickstoff erhöhen können.

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Table I. Amounts of nitrogen fixed in 35 days by *Nostoc punctiforme* (Kütz.) Hariot and *Scytonema bohneri* Schmidle per 100 ml culture solution

Forms	Type of culture	Dry weight of alga (mg)	Amount of nitrogen fixed (mg)			Nitrogen fixed as % of dry weight (%)
			In algal mat less in inoculum	In filtrate	Total	
Nostoc punctiforme	Pure	7.6	0.482	0.434	0.916	12.05
	Unialgal	–	3.806	0.871	4.677	–
Scytonema bohneri	Pure	19.8	0.703	0.284	0.987	4.98
	Unialgal	–	0.551	0.644	1.195	–

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