The results of mean HCN content in the leaves of the five varieties/hybrids at three stages of the crop growth are presented in Table I. It is seen that the initial level of HCN in all the samples is high ranging from 7·1 ppm to 55·5 ppm and there is a decrease in its content with advance in the age of the crop. The decrease is more marked between 25 and 50 days after sowing, especially in case of CSH–6 and CSH–1. It has been reported elsewhere that the HCN content was 94·5 mg%, 27·1 mg% and 11·7 mg% in 30, 45, and 90 day-old jowar fodder (on dry weight basis)[5]. Similar decrease was also noticed by other workers[2].

In the present study, it is also observed that there is a variation in HCN in the several varieties tested. Variety 168 has shown the lowest content of the constituent, followed by variety SB 101 in many samplings, even though the variety SB 1066 showed lower levels than SB 101, when the crop is 25 days old. However, hybrid CSH–6 has indicated the highest figures in all the three samplings. Varietal differences in HCN in the two varieties "Piper" and "Green leaf" Sudan grass and one hybrid Suhi-1 of sorghum were reported by other workers[6].

Data on the variation in HCN level in CSH–5, as affected by salinity, are given in Table II. It is noticed

TABLE II

Effect of salinity on HCN content (ppm) in leaves of sorghum (CSH–5) at various growth stages (on fresh weight basis)

Salinity levels ppm	Days after sowing			
	25	45	65	80
128 (Control)	39·8	5·3	1·3	0·4
1,280	28·4	6·7	2·1	0·4
2,560	25·8	6·8	2·1	0·8
3,840	24·4	10·6	1·6	..
5,120	23·7	11·8	1·6	0·7
7,680	13·7	13·7	1·6	0·8
10,240	13·7	13·7	2·7	1·4

that in the initial stage, HCN content in the leaves has been found to get reduced with increase in salinity from 39·8 ppm to 13·7 ppm. This initial reduction is due perhaps to the poor growth of the plants on account of salinity. The decrease, however, is not observed in the later samplings as the plants advance in age. It is also found that the initial level of HCN is high and decreases with age as observed earlier, irrespective of the salinity levels. But at lower salinity levels, the decrease is found to be sharp, especially between the first and second samplings unlike in the higher salinity series. Thus, while the levels of HCN at 25 and 45 days are 39·8 ppm, 28·4 ppm; and 5·3 ppm, 6·7 ppm in the control and salinity level of 1280 ppm respectively, the corresponding values for these two samplings at salinity levels of 3840 and 5120 ppm are 24·4 and 23·7 ppm; and 10·6 and 11·8 ppm. Very high salinity levels (7680 and 10240 ppm) do not show any variation in HCN levels in the first two samplings (13·7 ppm). It may be mentioned here that at high salinity levels, the vegetative growth period of the plant is found to be prolonged and hence, it is possible that the decrease in HCN content is not as marked as in the lower series of salinity. However, with further advance in growth, the differences among the treatments are not marked.

The studies, thus, reveal that there is difference in level of HCN in the sorghum varieties/hybrids tested and this is influenced by the vegetative growth period of the plant. A prolongation in vegetative phase of the crop by salinity is found to delay the reduction in the level of HCN in leaves.

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ISOLATION OF *SPIRILLUM LIPOFERUM* FROM THE STEMS OF WHEAT AND NITROGEN FIXATION IN ENRICHMENT CULTURES

Spirillum lipoferum has been found to be closely associated with roots of several grasses and crop plants including wheat[1-6]. In the present report, evidence is presented to point out that *S. lipoferum* could be isolated from the stems of several varieties of wheat by the enrichment culture method. Such enriched cultures were capable of fixing nitrogen to different

degrees depending on the varieties of the host used for enrichment. The stem portions of twenty dwarf, high yielding varieties of wheat grown on IARI farm were cut 5–8 cm above the ground level and their leaf sheaths were removed. They were then cut into 2–3 cm long pieces and surface sterilized with 70% C_2H_5OH followed by three successive washings each with sterile water and neutral phosphate buffer (0·15 M). The stem pieces were aseptically placed in sodium malate medium[4] in 10 ml screw cap tubes. Further enrichment and isolation were done by procedures described earlier[3]. *S. lipoferum* could be isolated from stem pieces of all the varieties of wheat as characteristic white sub-surface pellicles (Fig. 1).

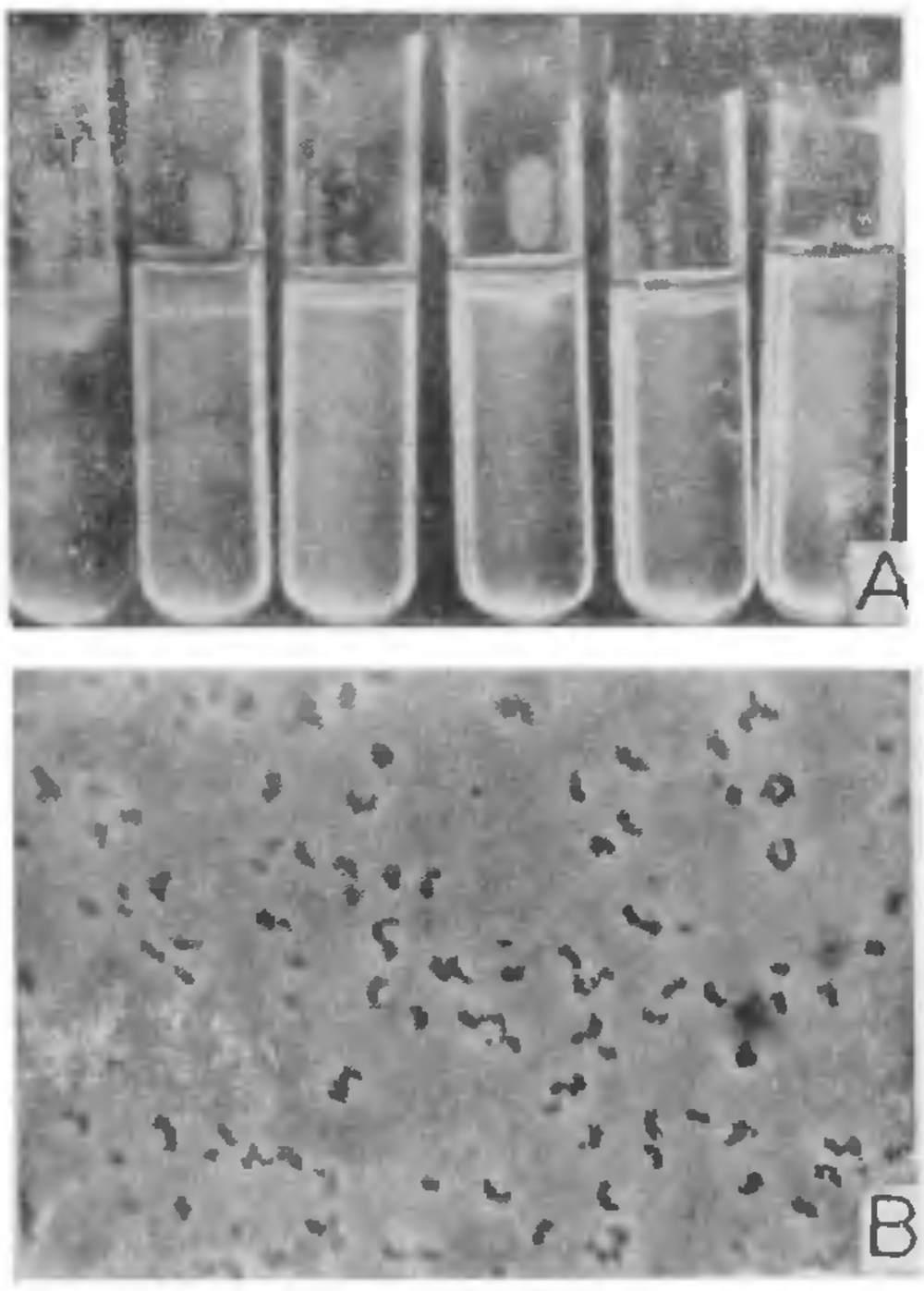

FIG. 1, A–B. Sub-surface pellicles of *S. lipoferum* isolated from 6 of the 20 different wheat varieties studied in the present investigation: from left to right—Raj. 1114; HD 2236; HD 2204; S 227; HD 2212 and HD 2009. B. Phase contrast photomicrograph of 48 hr old cultures of *S. lipoferum* isolated from the stem of wheat variety HD 2236 (× 2,000).

All the isolates were highly motile, showing characteristic cork screw-like motility and grew well on nitrogen-free sodium malate medium. The bacteria also exhibited rapid back and forth movement in straight line. The diameter of the cells ranged from 0·5–1·1 μm, with an average cell size of 0·8 × 2·3 μm, comparable to the one reported earlier for *S. lipoferum*[1,5]. However, the cells were smaller in size than those of *S. volutans*, the only type species of the redefined genus *Spirillum*[7]. Metachromatic granules were clearly seen in the slightly curved, rod-shaped cells. In some cultures, coccoidal cells were also observed.

The extent of nitrogen fixation was estimated by the Kjeldahl's procedure in enrichment cultures from 9 varieties of wheat in the following manner. Portions of stem pieces weighing 500 mg were surface sterilized and incorporated in 50 ml aliquots of sodium malate medium (without bromothymol blue) and incubated at 30° C for 14 days. Simultaneously, a series of control flasks containing stem pieces were autoclaved, incubated and assayed in a similar way for total nitrogen. The difference in the values for total nitrogen between the two series was taken as the amount of nitrogen fixed by the wheat stem due to enrichment with *S. lipoferum*. The results (Table I) showed that enrichment cultures obtained with surface sterilized stem pieces fixed varying amounts of nitrogen depending on the varieties and the native nitrogen content of stem pieces had no relationship with the ability of enrichment cultures to fix nitrogen. Among the varieties tested, UP 270 fixed the maximum amount of nitrogen (22·54 mg) whereas with WH 199, the fixation was only 5·70 mg N/50 ml.

TABLE I

Fixation of nitrogen in enrichment cultures of wheat stems in sodium malate medium (mean of 3 replicates)

Wheat varieties	mg N_2/50 ml of medium		
	Surface sterilized step pieces (A)	Autoclaved step pieces (B)	Nitrogen fixed (A − B)
S 227	17·36	3·42	13·94
S 308	15·09	4·27	10·82
HD 2160	18·78	3·06	15·72
HD 2216	16·51	3·67	12·84
HD 2236	14·77	1·96	12·81
WH 199	10·82	5·12	5·70
WH 209	14·23	1·71	12·52
UP 270	26·44	3·93	22·54
Raj. 1114	16·22	5·12	11·10
C.D. at 1%			4·51

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CHANGES IN NUCLEIC ACID LEVELS DURING AESTIVATION IN *PILA GLOBOSA* (SWAINSON)

CHANGES in enzyme activity levels were noted during aestivation in *P. globosa*[1,2]. Enzymes concerned with glycolysis were known to increase[3] whereas those concerned with TCA cycle decreased during aestivation[4]. The regulation of enzyme activity levels at the enzyme protein level was reported for some enzymes *vis-a-vis* phosphorylase by the interconversion of the two phosphorylases[5,6], glutamine synthetase by cooperative feedback inhibition[7]. But the control of enzyme activity level of many enzymes is regulated at the transcriptional level involving gene expression[8]. There were no studies so far concerning synthetic potential of aestivated snails to get a better understanding of aestivation and a study was undertaken to estimate the nucleic acids and RNA/DNA ratios in the three tissues, *viz.*, digestive gland, mantle and foot of active and 6 months aestivated *P. globosa*.

Collection, maintenance and mode of aestivation were described elsewhere. The tissues were isolated in cold. DNA was estimated by the method of Giles and Myers[9] using diphenylamine. RNA was estimated by the method of Munro and Fleck as described by Glick[10]. The values were expressed as μ gm nucleic acid/gm wet wt. of the tissue.

The results showed a decrease in the nucleic acid content in all the tissues of aestivated snail. The decrease in the DNA content was relatively less except in mantle where 32·7% decrease was observed. The DNA content decreased only to the extent of 11% and 25·5% in the case of digestive gland and foot respectively. The decrease of DNA in the digestive gland was statistically insignificant probably due to the pivotal role it plays in the metabolism of aestivated snail. The significant decrease of DNA content in the mantle and the foot might be due to a decrease in the cell count in these tissues due to the activity of intracellular lysosomal enzymes. The resulting proteins, fats and carbohydrates might be a source of nourishment to the tissue cells. Probably it is this capacity of the snail (in addition to maintain-

TABLE I

Levels of DNA and RNA fractions in the tissues of active and aestivated Pila globosa

(Values expressed in μ gm of nucleic acid/gm wet wt.) Each value is the average of 15 different estimations

Sl. No.	Nucleic acid fraction	Digestive gland		Mantle		Foot	
		Active	Aest.	Active	Aest.	Active	Aest.
1.	DNA	4517±260	4017±190	2789±125	1875±120	1623±350	1102±165
	% Change		−11·07		−32·77		−25·47
2.	RNA	7725±760	4300±376	3413±276	1944±194	1389±139	745±85
	% Change		−44·33		−43·04		−46·35
3.	RNA/DNA	1·71	1·07	1·22	1·04	0·86	0·68
	% Change		+37·44		+15·29		−20·9

Note: 1. ± indicates standard deviation.
2. The changes noted are significant at 0·001 level.