TABLE I

Effect of pH of the media on in vitro *root nitrate reductase activity*

pH	Nitrate reductase activity μ moles nitrite produced per hr^{-1}	
	g^{-1} fresh root wt.	Total fresh root wt. (g) per plant
4·0–4·5	2·06	43
5·0–5·5	1·68	34
6·0–6·5	3·70	53
7·0–7·5	4·89	43
7·5–8·0	2·56	12

TABLE II

Effect of pH of the media on NO_3^- uptake

Age of the plant (days)	pH 4·0–4·5	5·0–5·5	6·0–6·5	7·0–7·5	7·5–8·0
	mg NO_3^- N per plant				
Σ_{18}^{23}	126	113	28	18	10
$\bar{X}$	21	18·8	4·7	3·0	1·7
%	100	85·5	22·3	14·3	8·0
Σ_{9}^{23}	256	216	167	109	60
$\bar{X}$	17·1	14·4	11·1	7–3	4·0
%	100	84·4	65·2	42·6	23·4

% was calculated relative to values set at 100% obtained with plants grown at pH 4·0–4·5.

Σ_{18}^{23} cumulative NO_3^- uptake for the period 18th day to 23rd day (Total 6 days).

Σ_{9}^{23} cumulative NO_3^- uptake for the period 9th day to 23rd day (total 15 days).

$\bar{X}$ Mean NO_3^- uptake per day.

to protect the root NRA from various inhbitors (Wallace[7]). All samplings were done in triplicate and all assays were carried out at least in duplicate.

The data in Table I show that NRA (g^{-1} fresh weight basis) was about the same when plants were grown at pH 4·0 to pH 5·5 media. Activity increased as pH increased from 5·0 to 7·5 and decreased at pH 8·0 to 8·5. However, activity at pH 8·0 on g^{-1} fresh weight basis was higher than at pH 4·0–4·5. Total root activity per plant was highest when plants were grown at pH 6·0–6·5 and lowest at pH 8·0–8·5 (Table I). In the present studies, NRA was measured only at the end of the experiments. This was considered to be adequate as the authors have earlier observed uniform root NRA from 10 days after sowing to 20–23 days after sowing (data not reported in this note).

A comparison of NRA between pH treatment 4·0–4·5 and 7·0–7·5 reveals that roots grown at pH 7·0–7·5 possess at least double the activity over treatments at pH 4·0–4·5 on g^{-1} fresh weight basis (Table I). Total root activity per plant, however, is same. But the data from Table II show that treatment at pH 7·0–7·5 accumulated only 43% nitrate over treatment at pH 4·0–4·5, when the cumulative NO_3^- uptake for 15 days (between 9th and 23rd day after sowing) was considered. If cumulative uptake during the last six days was considered the treatment at pH 7·0–7·5 accumulated only about 14·3% nitrate over treatment at pH 4·0–4·5 (Table II).

These results indicate that NRA, expressed either on unit fresh weight or total plant root basis, has no relationship to NO_3^- uptake characteristics *per se*. Thus these data suggest that root nitrate absorption is independent of root NRA.

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OCCURRENCE OF *AZOSPIRILLUM* IN ASSOCIATION WITH THE ROOTS AND STEMS OF DIFFERENT CULTIVARS OF BARLEY (*HORDEUM VULGARE*)

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THE occurrence of *Azospirillum brasilense* as a micro-aerophilic non-symbiotic nitrogen fixer in association with the roots of rice, sorghum, maize and several grasses has been well documented[1,2,4,5]. The presence

of *Azospirillum* in stems of several varieties of wheat by an enrichment culture method has also been reported[3]. Beneficial response of barley to inoculation with *A. brasilense* under low levels of inorganic nitrogenous fertilizer application has also been reported[8]. As barley is grown mainly as a poor man's crop, under low input conditions this finding is of much significance and needs more detailed study regarding the occurrence of nitrogen fixing *Azospirillum* in association with the roots and aerial parts of the host in different cultivated varieties. With the recent emphasis over the production of hul-less types[7] by the breeders, this information is more essential for achieving higher yields. Therefore an attempt has been made to isolate *Azospirillum* from the roots and stems of the released varieties of barley along with those of recently developed hul-less strains and study the nitrogen fixation in enrichment cultures.

Eight varieties of barley as listed in Table I were used in the present study. All these varieties were grown during *Rabi* season of 1979–80 in replicated experiment in pot cultures under the same agronomic conditions of normal cultivation. The root portions of different varieties of barley were cut 5–8 cm below the ground level when the plants were 30 days old and the soil adhering to the roots was removed. The roots were then cut into 2–3 cm long pieces and the surface sterilised with chloramine T (1%) for 15 minutes followed by 3 successive washings with sterilised water and neutral phosphate buffer. The root pieces were aseptically transferred to sodium malate medium in 10 ml screw cap tubes. Further enrichment and isolation was done by procedures described earlier. *Azospirillum* could be isolated from all the varieties of barley as characteristic white sub-surface pellicles.

All the isolates were highly motile, showing characteristic spirillar movement and grew well in N_2-free sodium malate medium. The isolates were identified as *Azospirillum brasilense*[9].

The extent of nitrogen fixation by different isolates of *Azospirillum* was estimated by Kjeldahl's procedure. The results (Table I) showed that the isolates of *Azospirillum* from varieties 1845–10–5 and 1853–6–3 fixed 14–15 mg N/50 ml of sodium malate medium, while from Ratna, Jyoti and RDB–1 fixed 8–9 mg N/50 ml medium, and DL–3, DL–70 and DL–85 fixed 4·5–5·5 mg N/50 ml of sodium malate medium.

The extent of nitrogen fixation in enrichment culture from the stems of barley were also estimated in the *following manner : Portions* of stem pieces weighing 500 mg were surface sterilised with chloramine T (1%) for 15 min and incorporated in 50 ml aliquots of sodium malate and incubated at 35°C for a fortnight. Simultaneously, a series of control flasks *containing stem pieces were autoclaved at* 15 lb pressure for 15 min and incubated and assayed in a similar way for the total nitrogen. The differences in the values for total nitrogen between the two series were taken as the amount of N fixed by barley stems due to enrichment with *Azospirillum*. The results (Table I) showed that the enrichment cultures obtained with surface sterilised stem pieces were capable of fixing to different degrees on the varieties of host used for enrichment. The variation in the nitrogen fixing ability by different isolates may be attributed to variations in *Azospirillum* population in various varieties tested. Among the varieties tested, the variety 1853–6–3 fixed the maximum amount of N (20·2 mg), closely followed by 1845–10–5 which fixed 16·5 mg N/50 ml medium. With DL–85, a recently released variety the fixation was only 3·2 mg N/50 ml sodium malate medium.

TABLE I

Nitrogen fixation in root isolates and enrichment cultures of stems of different barley varieties

(Mean of three replicates)

Variety	Nitrogen fixation (mg N/50 ml sodium malate medium)	
	Root isolates	Enrichment cultures of stems
Hulled cultivars		
DL–3	4·5	5·2
DL–70	5·5	6·5
DL–85	4·5	3·2
Ratna	9·0	6·5
Jyoti	9·0	7·2
RDB–1	8·0	7·5
Recent Hul-less strains		
1845–10–5	14·0	16·5
1853–6–3	15·0	20·2

The varieties which fixed higher amounts of nitrogen are incidentally hul-less types and are reported to be of high protein content being derived from a complex cross involving Hyproly[7], a high protein and high lysine hul-less two-row barley identified from world barley collection in 1970[6]. It is not known, whether the high nitrogen fixing ability of these strains has resulted in high protein content of the grain. Further studies involving some more high protein types may throw some light in this direction.

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ASSIMILATION OF AMMONIA IN *AZOSPIRILLUM BRASILENSE* TARRAND *ET AL.*

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In all organisms the assimilation of nitrogenous compounds and their utilization in macromolecular synthesis are inevitable features in metabolism[1]. In nitrogen fixing diazotrophs, the atmospheric nitrogen is fixed in the cells as ammonia which is subsequently assimilated. Earlier studies with entric bacteria like *E. coli* and *Aerobacter* have revealed that although quite a few enzymes seem to be involved in the process of ammonia assimilation, glutamine synthetase (GS) plays a very pivotal role[2].

However, in most nitrogen fixing bacteria, ammonia assimilation proceeds through the coupled reactions of the dual enzymes, *viz.*, glutamine synthetase (GS) (E.C. 6.3.1.2) and glutamate synthase (GOGAT) (E.C. 2.6.1.53)[3]. Other enzymes like glutamate dehydrogenase (GDM) (E.C. 1.4.1.4) and glutamate asparate amino transferase (E.C. 2.6.1.12) have also been implicated in organisms like *Rhizobium*, *Bacillus polymyxa*, *Klebsiella*[4] etc. There appears to be little information available on ammonia assimilation in *Azospirillum*, the associative symbiotic nitrogen fixing diazotroph and the present study is an attempt in this direction.

Pure cultures of *Azospirillum brasilense* Tarrand *et al.* isolated from the root tissues of maize (*Zea mays* L.) and maintained on malate semisolid medium[5] was transferred to 100 ml of the malate broth taken in 250 ml Erlenmeyer flasks. Malate medium containing (i) 0·1% sodium citrate, (ii) 0·1% ammonium sulphate were also used.

The sterilised media were inoculated with a standardised cell suspension (*ca.* 10^6 cells/ml) of *Azospirillum* and the flasks were incubated at static conditions for seven days at 28±2° C. The cells were harvested by centrifugation at 10,000 × g for 15 min and the pellets were suspended in 5·0 ml of 0·05 M phosphate buffer (pH 7·0). The cells were sonicated at 0° C and the cell lysate was obtained by centrifugation at 40,000 × g for 10 min in a refrigerated centrifuge. The supernatant served as the enzyme source[6]. The cells at the time of harvest had sufficiently high nitrogenase activity (1620–2280 n moles of C_2H_4/mg protein/hr).

The enzymes, glutamine synthetase, glutamate synthase and glutamate dehydrogenase were determined by following the procedures of Shapiro and Stadtman[7] and Vandecasteele *et al.*[8]. All assays were performed for one hour at 37° C. The dependency of NADH or NADPH was also tested for the enzyme GOGAT. The results are presented in Table I.

The results have indicated that *Azospirillum* cells grown in malate medium without any nitrogen source recorded the maximum activity of glutamine synthetase. Also, the NADH and NADPH dependent glutamate synthase activity was evidently high. In nitrate and ammonia grown cell also the activity of GS and GOGAT was quite appreciable. However, no GDH activity could be detected in malate grown cells, while it was appreciable in nitrate and ammonia grown cells.

Studying the mechanism of ammonia assimilation, Tempest *et al.*[3] recently elucidated the operation of another pathway in microorganisms. It involves the amidation of endogenous glutamate to glutamine and subsequently reductive transfer of glutamine-amide nitrogen to the second position of 2-oxoglutarate producing a net synthesis of two moles of glutamate. Recently, Nagatani *et al*[9]. also presented evidence that glutamine synthetase occurs in diazotrophs in a predominantly poorly adenylated form characterised by a low km value for ammonia. In this form the enzyme is ideally suitable for nitrogen fixing organisms as it assures efficient assimilation of fixed nitrogen and prevents the accumulation of ammonia which inhibits