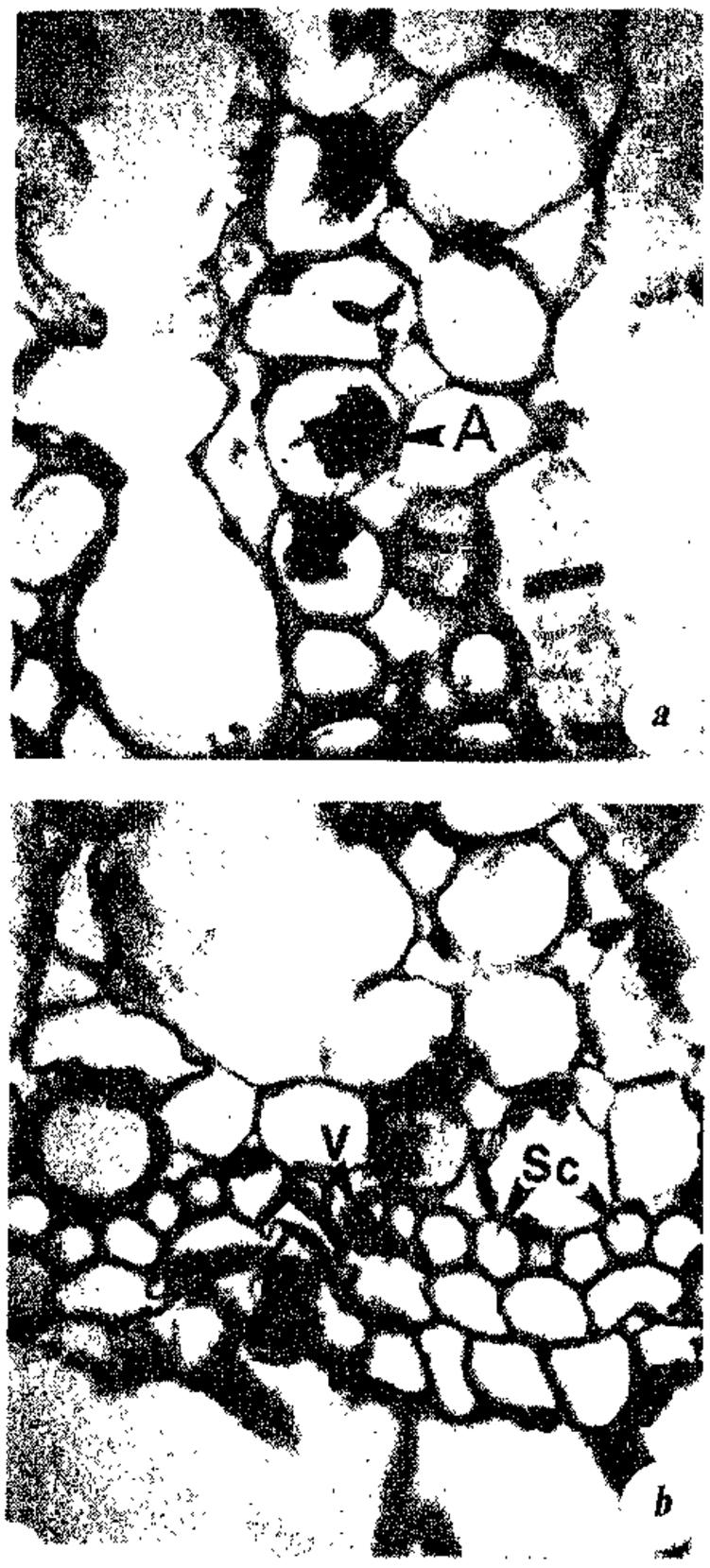
inoculation sand : soil mixture containing root segments of *Panicum maximum* colonized by *Glomus fasciculatum* was used and uninoculated plants served as control. Plants were harvested on the 60th day. Root systems were rinsed with running water, fixed and processed for light microscopic observations. Thin sections (7 μm) were stained for polysaccharides by periodic acid Schiff's (PAS) method[4].

The fine structure revealed that the mycorrhizal root cells were large, well defined, and filled with arbuscules, the site of nutrient exchange (Figure 1*a*). During the process of digestion, the dichotomously branched arbuscules were slowly degraded leading to clump formation, which finally disappeared.

It was observed that the mycorrhizal roots were considerably large and the cells were densely PAS positive compared to non-mycorrhizal roots indicating higher polysaccharide deposition in the cell walls. The most interesting observation was the penetration of lignified sclerenchymatous exodermal cells by VA mycorrhiza (Figure 1*b*). As far as we are aware, this is the first report of such penetration. This suggests that mechanical barrier of the lignified sclerenchymatous cells does not interfere in the establishment of the endophyte, thus upholding the recent observation of VA mycorrhiza in the lignified xylem vessels of ginger[5].



rse section of the mycorrhizal root showing: ›uscules (A) (×400); and *b*, sclerenchymatous V) (×400).

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External adsorption of *Azospirillum lipoferum* strain D-2 to plant roots and its effect on plant growth

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Inoculation of a strain of *Azospirillum lipoferum* D-2, isolated from roots of *Digiteria*, on barley crop resulted in an increase in dry weight and grain yield of the barley crop. Levels of total nitrogen in different parts of the plant also increased in response to *Azospirillum* inoculation. Another strain of *Azospirillum lipoferum* M-2, isolated from maize, also showed similar response on barley crops suggesting non-specificity of strains for the host plants. Events leading to the colonization of *Azospirillum lipoferum* D-2 on wheat roots have been studied and described using scanning electron microscopy.

IN any given soil–plant environment, a particular type of microfloral population gets established by the compound(s) excreted by the roots, which serve as carbon and energy sources for these microorganisms. Soil is an extremely complex environment, making it very difficult to introduce and establish large numbers of laboratory cultured beneficial organisms. Some of the most promising organisms, capable of colonizing roots in large numbers and exerting beneficial effects on plants, belong to genus *Azospirillum*[1]. They colonize mainly forage and grain grasses and are readily isolated from the rhizosphere[2].

There are few indications for the type of factors contributing to plant–bacterium specificity in such associations[3,4]. Plant growth responses observed after inoculation of *Azospirillum* have been explained by

nitrogen fixation[5], phytohormone production[6] and enhanced nutrient uptake[7] by these bacteria. Evidences are presented in the present study for external adsorption of *Azospirillum lipoferum* to plant roots and its effect on plant growth stimulation.

A. lipoferum strain D-2 was a local isolate obtained from the surface-sterilized roots of *Digiteria*. Growth conditions for *A. lipoferum* D-2 were the same as described earlier[8]. The level of IAA in the culture filtrates was estimated by the colorimetric assay of Gordon and Weber[9]. Bacteria (washed suspension in phosphate buffer) were directly inoculated on the roots of 3-day-old seedlings grown on wet filter paper in petri dishes. Twenty four hours after inoculation, roots were treated as follows: (i) rinsed gently with sterile distilled water, (ii) surface disinfected with 1% (v/v) NaOCl for 5 min and washed with water. Root pieces after glutaraldehyde fixation, dehydration and critical point drying were stuck to stubs, coated with gold–palladium and examined by a Zeol JSM-T300 scanning electron microscope at 15 kV voltage.

Plant experiments in field were conducted at the Indian Agriculture Research Institute, New Delhi.

A. lipoferum D-2 showed high acetylene reduction activity under microaerophilic conditions[10]. If it is assumed that the principal factor responsible for increased growth and nitrogen yield of various cereals is due to nitrogen-fixing ability of the microorganisms interacting with plants, *A. lipoferum* D-2 could serve as a potential inoculant, owing to its nitrogen-fixing ability under cultured conditions. Plant inoculation experiments were conducted at different levels of fertilizer nitrogen. As is evident from Table 1, inoculation with *Azospirillum* resulted in an increase in dry weight and grain yield of barley crop. Similarly, inoculation increased the number of ear per pot, number of grain per ear and grain weight per ear. Levels of total nitrogen (%N) in straw, husk and grains increased in response to *Azospirillum* inoculation. Yields obtained at the inoculated intermediate levels of N fertilization were higher than in fully fertilized uninoculated plants. Another strain of *A. lipoferum* isolated from maize, strain M-2, also gave similar response on inoculation suggesting that these strains were not host-specific and positive response could be obtained with several crops.

Increase in agriculturally significant yield through *Azospirillum* inoculation does not seem to be confined to their role in nitrogen fixation, but may involve influence of phytohormones on roots or enhanced nutrient uptake by roots[5–7]. Production of indole compounds was checked in *A. lipoferum* D-2. IAA excretion was similar under both nitrogen fixing and non-fixing conditions (Table 2). However, excretion of IAA by ammonium grown culture could be stimulated by the addition of tryptophan. Although *A. lipoferum* D-2 produced some IAA, production was not sufficiently high to promote the observed plant-growth responses. *A. brasilense* has been shown to produce much higher quantities of IAA (50 μg/ml)[11].

According to Patriquin *et al.*[12], the greater efficiency and magnitude of positive responses to inoculation with *Azospirillum* than with other growth-promoting bacteria are related to (i) its being a very effective colonizer of roots and, (ii) to nitrogen fixation. Colonization of roots was studied in axenically grown wheat inoculated with *A. lipoferum* D-2. Inoculation resulted into colonization of mucilagenous sheath surrounding root hair (Figure 1*a*), whereas root hair surface of uninoculated plants was smooth and non-mucilagenous (Figure 1*b*). Some root hair became deformed in response to *Azospirillum* inoculation. The colonization started from the tip of the root hairs and gradually extended towards the base. Attachment of *Azospirillum* cells to root was polar and associated with the presence of granular and fibrillar material (Figure 1*c*), which was not seen in the uninoculated roots (Figure 1*d*). Disinfection with NaOCl of highly colonized roots eliminated most of the bacteria (Figure 1*e*).

Table 1. Effect of inoculation of *A. lipoferum* strains D-2 and M-2 on barley crop*.

Growth condition	Dry wt of straw (g)	Dry wt of grain (g)	No. of ears per pot	Grain wt per ear (g)	No. of grain per ear	% Nitrogen of straw	%Nitrogen of husk	%Nitrogen of grain
Control	11.07	09.95	08.25	1.11	38.00	1.41	0.73	2.21
Azospirillum	20.57	16.00	13.25	1.07	35.50	1.74	0.85	2.28
80 kg N	22.97	30.70	15.50	2.08	52.00	2.11	0.70	2.10
40 kg N	30.62	25.60	11.25	2.54	50.00	2.10	0.83	2.20
26.6 kg N	19.40	19.07	11.50	1.68	49.00	2.10	0.74	2.16
26.6 kg N + *A. lipoferum* D-2	25.40	23.30	17.25	1.35	38.75	1.99	0.76	1.95
40 kg N + *A. lipoferum* D-2	38.50	37.37	23.25	1.61	70.50	2.10	1.01	2.49
26.6 kg N + *A. lipoferum* M-2	22.62	22.77	12.00	1.50	60.00	1.44	0.81	2.51
40 kg N + *A. lipoferum* M-2	42.62	34.62	19.25	2.16	64.25	2.20	1.06	2.69

*Results are mean of five independent experiments.

Table 2. Excretion of indoleacetic acid (IAA) by *A. lipoferum* D-2 under different growth conditions.

Growth condition	IAA (μg/ml)
Nitrogen grown	0.35
Ammonium (10 mM) grown	0.28
Ammonium (10 mM) + tryptophan (1 mM) grown	2.55

Adsorption of bacteria may give the rhizosphere bacteria nutritional and favourable microspace advantages. Rhizosphere bacteria may adsorb as single cells or as aggregates, but usually are not scattered randomly[12]. *Azospirillum* cells were found in intercellular spaces of the cortex of various cereals, grown

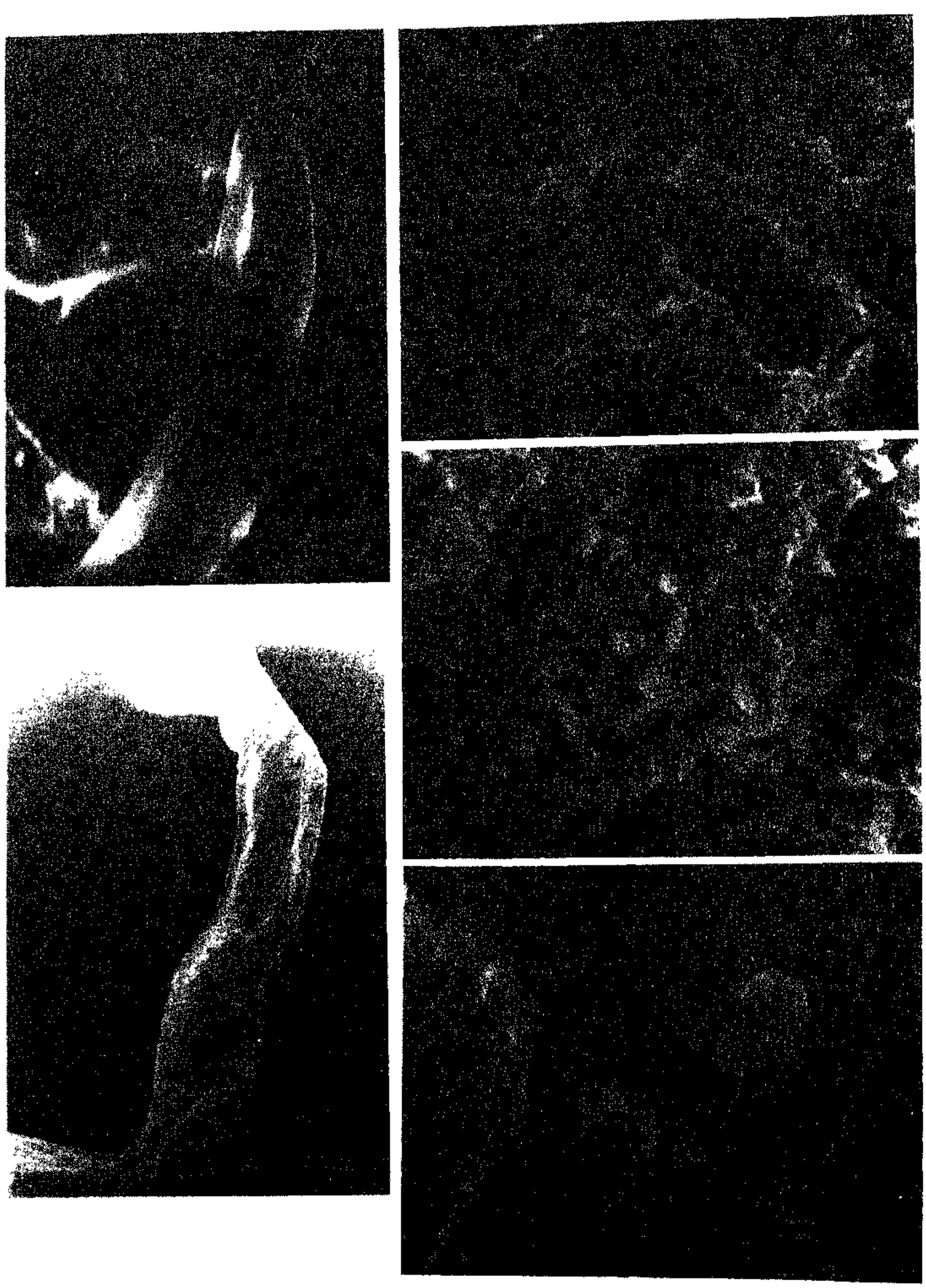

Figure 1. Scanning electron micrographs of root hair from: *a*, uninoculated wheat (×2,300)—no mucoid surface or deformation visible; *b*, inoculated wheat (×4,200)—change in surface texture and root hair curling can be seen; *c*, uninoculated wheat (×6,000)—no bacterial attachment or fibrillar mucigel can be seen; *d*, inoculated wheat (×6,000)—showing polar attachment of bacteria (indicated by arrow) to a mucilagenous fibrillar mass on root surface, and *e*, disinfected 24 h after *Azospirillum lipoferum* D-2 inoculation. Scanty bacteria but mucilagenous mass is visible on the root surface (×5,800).

either axenically or under field and greenhouse conditions as well as within dead cortex cells and root hairs[11].

This study reveals that beneficial effect of *Azospirillum* inoculation can be attributed to its nitrogen-fixing ability in both free-living as well as associative conditions. Non-specificity for the host makes *Azospirillum* a potential inoculant for several agronomically important crops. It is also evident from the present results that external adsorption of *A. lipoferum* D-2 is rather weak. Adsorption of bacteria to plant, either passive or active, depends on the metabolism of both organisms. Preferance of *A. lipoferum* D-2 to utilize dicarboxylic acids such as malate and succinate[13], suggests that accumulation of these dicarboxylic acids (in C-4 plants), and their transport to the roots, in which these bacteria reside, favours nitrogen fixation.

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Biochemical changes in germinating triticale in response to pretreatment with pyridoxine

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The present paper reports some biochemical changes in germinating triticale in response to pre-treatment of grains with 0.0 (control), 0.001, 0.01 and 0.1% aqueous solutions of pyridoxine hydrochloride. These treated seeds were sand-cultured in a B.O.D. incubator and analysed at 12, 18 and 24 h of their germination. Among the three concentrations of the vitamin used, 0.01% proved most effective in increasing the levels of α-amylase, catalase and peroxidase and solubilizing the reserve food of the germinating grain.

THE seed being a dispersal unit, is equipped with structural and physiological devices and is well provided with food reserves which sustain the young plant until an autotrophic organism can be established. However, impaired partitioning of photosynthates and other substances during seed formation result in low germinability of the seeds[1]. The vitamins of B-group have been variously claimed to improve germination when administered into the seeds through soaking in their dilute solutions[2-5].

Triticale, an intergeneric hybrid of wheat and rye, has poor germination in the field[6]. In our earlier findings we established that pretreatment of grains with some B-vitamins significantly improved the germination in this crop[7]. Vitamin administered in the seed triggered some hitherto unknown physiological processes which led to the early emergence of radicle. The study of the biochemical and physiological phenomena that occur in a germinating seed and activities that are uniquely related to mobilization of reserve food have been discussed here.

The seeds of triticale var. Tigre"s" were obtained from CIMMYT, Mexico and multiplied at Aligarh. The healthy grains of the previous year were surface sterilized with 0.01% $HgCl_2$ solution. Thereafter, seeds were washed with de-ionised water and soaked for 8 h in 0 (control, T_1), 0.001 (T_2), 0.01 (T_3) or 0.1% (T_4) aqueous solutions of pyridoxine hydrochloride. These pretreated grains were allowed to germinate in a B.O.D. incubator on the surface of sterile sand spread on a petri dish of 15 cm diameter in the dark at 27±2°C. These seeds were taken out at intervals of 12, 18 and 24 h of germination.

The α-amylase activity was measured colorimetrically using the method based on the starch–iodine reaction forming starch–iodide complex[8]. Catalase activity was measured titrimetrically[9] whereas, peroxidase activity was estimated on colorimeter using purpurogallin for standard curve[9]. However, the determination of carbohydrate and protein and pyridoxine contents was carried out colorimetrically[10-12].

Irrespective of the treatment, pyridoxine level in the grains increased as the germination progressed but the increase was very prominent in the grains soaked in vitamin solution. The order of the effectivity of the treatment was found to be $T_4 > T_3 > T_2 > T_1$ (Figure 1*a*). It expressed a direct correlation between the levels