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.

1. Dobereiner, J. and Day, J. M., in *Proceedings of the First International Symposium on Nitrogen Fixation* (eds Newton, W. E. and Nyman, C. J.), Washington State University Press, Pullman, 1976, p. 578.
2. Partriquin, D. G., Dobereiner, J. and Jain, D. K., *Can. J. Microbiol.*, 1983, **29**, 900.
3. Mandimba, G., Heulin, T., Bally, R., Guckert, A. and Balandreau, J., *Plant Soil*, 1986, **90**, 129.
4. Reinhold, B., Hurek, T. and Fendrik, I., *J. Bacteriol.*, 1985, **162**, 190.
5. van Berkum, P. and Bohlool, B. B., *Microbiol. Rev.*, 1980, **44**, 481.
6. Tien, T. M., Gaskins, M. H. and Hubbell, D. H., *Appl. Environ. Microbiol.*, 1979, **37**, 1016.
7. Lin, W., Okon, Y. and Hardy, R. W. F., *Appl. Environ. Microbiol.*, 1983, **45**, 1775.
8. Saxena, B., Modi, M. V. and Modi, V. V., *J. Gen. Microbiol.*, 1986, **132**, 2219.
9. Gordon, S. A. and Weber, R. P., *Plant Physiol.*, 1951, **26**, 192.
10. Saxena, B. and Modi, V. V., *Curr. Microbiol.*, 1989, **18**, 231.
11. Hartmann, A., Singh, M. and Klingmuller, W., *Can. J. Microbiol.*, 1983, **29**, 916.
12. Dazzo, F. B. and Hollingsworth, R. F., *Biol. Cell*, 1984, **51**, 916.
13. Saxena, B., Parikh, R. H. and Modi, V. V., *Indian J. Exp. Biol.*, 1988, **26**, 657.

ACKNOWLEDGEMENT. Babita Saxena thanks Council of Scientific and Industrial Research, New Delhi for a fellowship.

16 May 1989

Biochemical changes in germinating triticale in response to pretreatment with pyridoxine

I. Haque and A. Ahmad
Department of Botany, Aligarh Muslim University, Aligarh 202 002, India

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

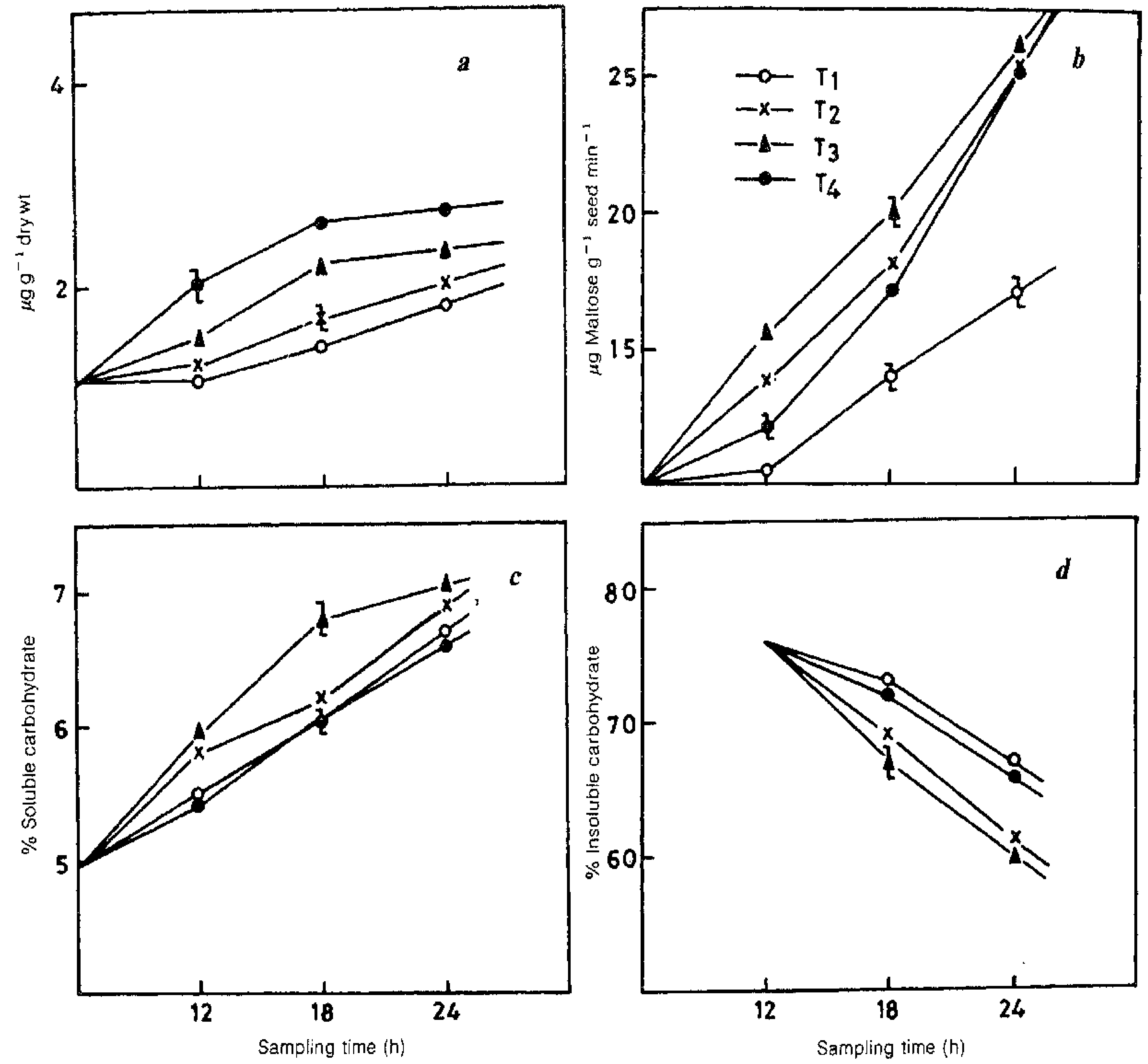

Figure 1. Effect of pyridoxine on *a*, seed pyridoxine content; *b*, α-amylase activity, *c*, soluble carbohydrate content; and *d*, insoluble carbohydrate content of germinating seeds of triticale.

of the vitamin in the grains and that of the soaking solution. It may, therefore, be proposed that uptake of the vitamin by the grain is a physical rather than a physiological process. The increase in vitamin content during grain germination has also been reported in cereals and legumes[13,14]. During germination, vitamin is either released from its bound form or synthesized *de novo*[15].

It is generally agreed that during germination of the seed, the developing embryo withdraws nutrients in the soluble form from the stored quota in the seed. Starch, the reserve carbohydrate, is acted upon by α-amylase to hydrolyse them to a simpler carbohydrate (glucose), which is easily metabolized by the growing embryo. The synthesis of the enzyme in the aleurone cells, under normal conditions is limited by gibberellic acid secreted by embryo or scutellum. The level of α-amylase in the grain may, however, be elevated by soaking them in pyridoxine solution (Figure 1*b*). The lower concentrations of vitamin (0.001 and 0.01%) were more effective than the higher concentration (0.1%) which probably proved to be sub-optimal. Similarly[16], other vitamins (riboflavin and niacin) also elevated the level of α-amylase in the tomato seedlings raised from treated seeds through increase in gibberellic acid content. Another factor responsible for the increase in α-amylase activity might have been the involvement of pyridoxine as a co-factor of aminotransferases[17], responsible for the synthesis of amino acids, the building block of protein.

It may, therefore, be suggested that pyridoxine either partly replaced gibberellic acid requirement or accelerated its synthesis to induce the production of additional amount of enzymatic protein. This will naturally speed up the rate of hydrolysis of carbohydrate. Therefore, the soluble carbohydrate content in the germinating grains increased (Figure 1*c*), whereas, the level of insoluble carbohydrate subsequently decreased (Figure 1*d*).

The activity of other enzymes, catalase and peroxidase, is very much associated with the presence of glyoxysomes[18] responsible for gluconeogenesis of lipids in the aleurone layer cells. The levels of these enzymes increase as the germination advances[19,20]. However, this increase was much more prominent in the grains treated with pyridoxine especially when soaked in 0.01% solution of vitamin (Figures 2*a*, *b*). The involvement of pyridoxine in the form of pyridoxal phosphate as a co-factor of δ-amino levulinic acid synthetase in the synthesis of catalase, a porphyrin protein[21] may be the reason to defend such an increase in the catalase activity in response to pyridoxine application. As regards increased production of peroxidase in the treated seed, nothing could be said definitely. The other vitamins (B_1, B_2 and C)[22] have significant effect on catalase activity in cotton leaves raised from pre-treated grains.

At the onset of the germination the solubilization of reserve protein started, therefore, the levels of soluble protein increased and that of insoluble protein decreased. But this process, like the solubilization of carbohydrate, was very much facilitated in grains soaked in pyridoxine solution (Figures 2*c*, *d*), 0.01% of the vitamin proved most effective like other parameters,

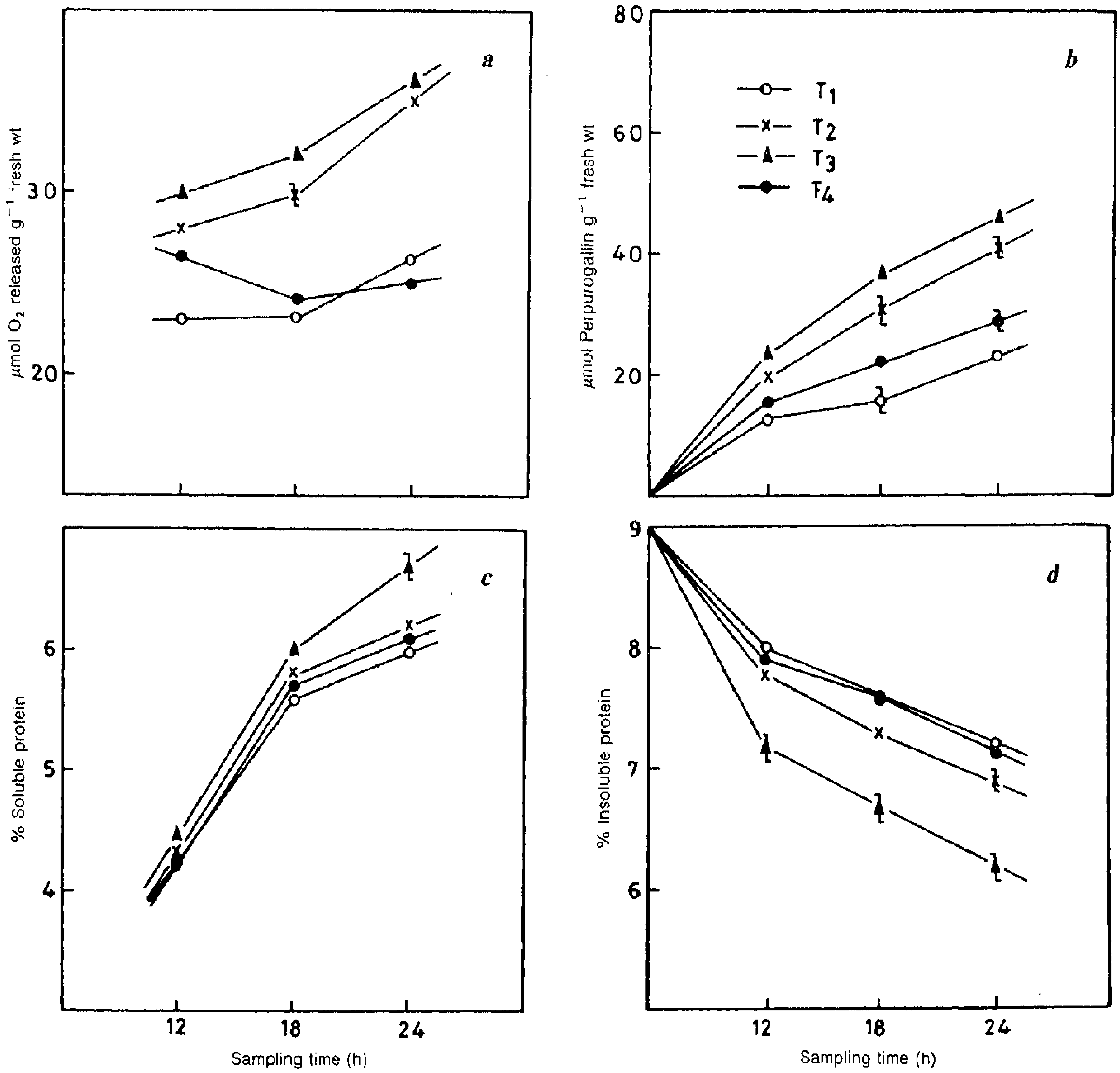

Figure 2. Effect of pretreatment of grains with pyridoxine on *a*, catalase activity; *b*, peroxidase activity; *c*, soluble protein content, and *d*, insoluble protein content during germination.

whereas, 0.1% being supra-optimal did not prove beneficial and the lowest concentration of the vitamin (0.001%) tried went unnoticed. The synthesis of additional amount of protein from liberated amino acids also accounted for the increased level of soluble protein. Further beneficial effect of pyridoxine in the process of seed germination is not altogether surprising as vitamin is a coenzyme in amino acid synthesis[17], where organic acids produced during oxidation of carbohydrate in Kreb's cycle are used.

1. Strognov, B. P. and Genkel', K. P., *Sov. Plant Physiol.*, 1976, **23**, 164.
2. Iijima, T., *J. Hort. Assoc. Jpn.*, 1952, **21**, 117.
3. Aizikovick, L. E., *Tr., Dnepropetr. Skh. Inst.*, 1967, **9**, 66.
4. Ovcharov, K. E. and Kulieva, L., *Khlopkovodstvo*, 1968, **17**, 41.
5. Serebryakova, N. V., *Hortic. Abstr.*, 1973, **43**, 2990.
6. Inam, A., Ph.D. thesis, 1978, Aligarh Muslim University.
7. Haque, I., Ahmad, A. and Muzaffar, S. S., *J. Indian Bot. Soc.*, 1988, **67**, 225.
8. Jones, R. L. and Varner, J. E., *Planta (Berl.)*, 1967, **72**, 155.
9. Chance, B. and Maehly, A. C., *Methods Enzymol.*, 1955, **1**, 764.
10. Dubois, M., Gills, K. A., Hamilton, J. K., Robers, P. A. and Smith, F., *Anal. Chem.*, 1956, **28**, 350.
11. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
12. Hochberg, M., Melnick, D. and Oser, B. L., *J. Biol. Chem.*, 1944, **155**, 119.
13. Burkholder, P. R. and McVeigh, I., *Proc. Natl. Acad. Sci., U.S.A.*, 1942, **20**, 276.
14. Burkholder, P. R., *Science*, 1943, **97**, 562.
15. Dagis, K. I., *Sov. Plant Physiol.*, 1959, **6**, 437.
16. Rao, P. G., Jayasree, G. and Kodandaramaiah, J., *Curr. Sci.*, 1981, **50**, 953.
17. Lehninger, A. L., in *Principles of Biochemistry*, Worth Publishers, New York.
18. Jones, R. L., *Planta*, 1971, **103**, 95.
19. Ito, S. and Hayashi, S., *Nippon Sakumotsu Gakkai Kiji*, 1961, **30**, 97.
20. Palmiano, E. P. and Juliano, B. O., *Plant Physiol.*, 1973, **52**, 274.
21. Kikuchi, G., Kumar, A., Talmage, P. and Shamin, D., *Biol. Chem.*, 1958, **233**, 1214.
22. Milieva, D., *Crop Abstr.*, 1971, **24**, 3993.

ACKNOWLEDGEMENT. We are grateful to Prof. M. M. R. K. Afridi, for his keen interest and valuable suggestions.

13 July 1989

Auxin–phenol-induced rooting in a mangrove, *Rhizophora apiculata* Blume

K. Kathiresan, G. A. Ravishankar* and L. V. Venkataraman*†
CAS in Marine Biology, Annamalai University,
Porto Novo 608 502, India
*Autotrophic Cell Culture Discipline, Central Food Technological Research Institute, Mysore 570 013, India

The hypocotyls of *Rhizophora apiculata* responded well to auxin and phenol treatments for rooting. Treatment of hypocotyls with indole-3-acetic acid (IAA) (0.05 mg/l) promoted root length by 1.9-folds over the control. The phenolic acid and IAA synergism was well pronounced in root initiation rather than in root elongation. Of the phenols studied, 1,3,5-benzenetriol in combination with IAA induced 16 roots per hypocotyl as against 10 in control. The rooted seedlings were easily established in the soil.

RHIZOPHORA APICULATA is a common mangrove species, which propagates through viviparous seedlings. The hypocotyls often exhibit poor rooting resulting in inefficient establishment of seedlings in loose and muddy soil of the coastal environment[1]. To overcome this, an attempt was made to enhance the rooting of the hypocotyls by application of auxins and phenolic compounds.

Healthy hypocotyls of *Rhizophora apiculata* Blume. with length of 27 ± 2 cm were collected from an individual plant in April 1989 from the Pichavaram mangroves (11°27′N, 79°47′E), Tamil Nadu. The hypocotyls were placed separately in 500 ml beakers containing 250 ml of test medium. The experimental media used were prepared using seawater (salinity 15 g/l) with auxins—indoleacetic acid (IAA) and indolebutyric acid (IBA) at 0.05, 0.5, 1.0, 2.0, 5.0, 10.0 mg/l each. Phenolic compounds—caffeic acid, catechol, ferulic acid, gallic acid, 1,3,5-benzenetriol, salicylic and tannic acids (Sigma Chemicals, USA)—were administered at 1, 10, 100 mg/l with or without IAA (2 mg/l). All media were adjusted to pH 7. Four replicates were taken for each treatment. The control received seawater alone. The beakers were wrapped with black paper and incubated in diffused light at ambient temperature of 27 ± 2°C. After 15 days of incubation, root growth characteristics were recorded.

IAA and IBA enhanced root length at lower concentrations with range of 1.3- to 1.9-fold (Figures 1 and 2). However, the increase in the number of roots was not significant. IAA at 0.05 mg/l greatly promoted root length by 1.9-fold compared to the control. Higher concentrations of IBA (2, 5, 10 mg/l) were found to have inhibitory effect on rooting (Figure 2).

---

†For correspondence.