daries and tertiaries) might have probably suffered giberellin insufficiency leading to the reduction in length, thereby causing an increased spikelet density and clustering of spikelets in rice.

19 April 1986; Revised 1 August 1987

1. Sreedharan, P. N. and Mishra, R. N., *SABRAO Newsl.*, 1973, **5**, 141.
2. Pavithran, K., *Crop improvement research*, (eds) T. C. Yap, K. M. Graham and J. Sukaimi, SABRAO, Kuala Lumpur, 1983.
3. Futsuhara, Y., Kondo, S., Kitano, H. and MII, M., *Jpn. J. Breed.*, 1979, **29**, 151.
4. Ramiah, K. and Rao, M. B. V. N., *Rice breeding and genetics*, ICAR, New Delhi, 1953.
5. Butany, W. T. and Seetharaman, R., *Curr. Sci.*, 1960, **29**, 188.
6. Seetharaman, R., *Rice genetics and cytogenetics*, IRRI, Philippines, 1964.
7. Takahashi, M., *Rice genetics and cytogenetics*, IRRI, Philippines, 1964.
8. Richharia, R. H., *ORYZA*, 1979, **16**, 1.
9. Pavithran, K., *International rice genetics symposium*, IRRI, Philippines, 1985.

SEEDS OF GRAMINACEOUS PLANTS AS CARRIERS OF *AZOSPIRILLUM*

A. V. RAO and B. VENKATESWARLU
Division of Soil-Water-Plant Relationship, Central Arid Zone Research Institute, Jodhpur 342 003, India.

THE nitrogen-fixing microaerophilic bacterium, *Azospirillum* has been receiving much attention owing to its association with the roots of many agronomically important graminaceous plants[1-4]. Inoculation with this organism was found to significantly increase production of dry matter and grain yield of several crops like maize, wheat, sorghum and pearl millet[2,3,5,6]. Although this bacterium has been isolated from the roots of several monocotyledonous and dicotyledonous plants, it was found to be more commonly associated with the roots of graminaceous plants which release more carbon-rich compounds in the rhizosphere in the form of root exudates. Besides roots, this organism was also associated with the stem region of certain plants[7]. In the present paper we report the isolation of *Azospirillum* from the seeds of many graminaceous plants and discuss its implications.

Seeds of several graminaceous and non-graminaceous plants (table 1) were subjected to brief surface sterilization (by immersing in 1% sodium hypochlorite solution for 10 min) followed by thorough washing with sterile water. After soaking the seeds in sterile water, the seed coat was broken and these seeds were transferred aseptically into test tubes, containing nitrogen-free-semi-solid malate medium[8], each of 3 seeds in the case of large seeded and 6 in the case of small seeded crops with 5 replicates. After 2 days of incubation at 30 ± 1°C, pellicle-like growth appeared below the surface of the medium. The nitrogenase activity of these cultures was assayed by acetylene reduction technique[4]. From all the tubes which showed acetylene reduction, the growth was sub-cultured 4 to 5 times in the same medium and finally a loopful of the growth was diluted and plated on potato sucrose agar medium. After 10 days of incubation, the dry wrinkled pink colonies were transferred to the semi-solid malate tubes. These were confirmed as *Azospirillum* from the formation of typical subsurface pellicle, nitrogenase activity and characteristic spiral movements of the cells[9].

The seeds which were found to carry *Azospirillum* were surface-sterilized as above and sown in test tubes containing sterilized sand + vermiculite mixture (1:1) to find out whether the organism from the seeds gets established in the roots upon germination. The root pieces of one-week-old seedlings were placed aseptically in nitrogen free-semi-solid

Table 1 *Isolation of Azospirillum from the seeds of selected plants*

Name of the plant	N_2–ase activity* of seed enrichment cultures	Presence of *Azospirillum*
Graminaceous		
Wheat (*Triticum aestivum*)	688.2	+
Pearl millet (*Pennisetum americanum*)	523.8	+
Barley (*Hordeum vulgare*)	29.6	+
Oats (*Avena sativa*)	75.2	+
Sorghum (*Sorghum vulgare*)	169.6	+
Proso millet (*Panicum miliacaum*)	–	+
Foxtail millet (*Setaria italica*)	–	+
Cenchrus ciliaris	33.8	+
Lasiurus sindicus	50.7	+

*nmol C_2H_4/seed/day.

malate medium and the tubes were observed for the growth of *Azospirillum* after 48 h. The acetylene reduction of these enrichment cultures was determined and the presence of *Azospirillum* was confirmed as described above.

Azospirillum could be isolated in pure culture from the seeds of pearl millet, wheat, barley, oats, sorghum, *Cenchrus ciliaris* L. and *Lasiurus sindicus* L., proso millet and foxtail millet. However, the seeds of none of the non-graminaceous plants like cowpea [*Vigna unguiculata* (L.) Walp.], mung bean [*Vigna radiata* (L.) Wilczek], cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.], moth bean [*Vigna aconitifolius* (Jacq.) Marechal], and *Tephrosia purpurea* L. and *Sesamum indicum* L. harboured this bacterium. Though bacterial growth was observed with all the seed samples within 48 h, only the enrichment cultures from seeds of graminaceous plants showed acetylene reduction. The nitrogenase activity of the seed enrichment cultures varied from 29.6 to 688.2 nmol of C_2H_4/seed/day and the maximum was observed with wheat followed by pearl millet (table 1). Microscopic observation of all the seed enrichment cultures which showed nitrogenase activity revealed a large number of actively moving *Azospirillum* cells. These were isolated in pure culture and authenticated following the characters described by Tarrand *et al*[9]. However in the case of proso millet and foxtail millet the cells were very few, and the enrichment cultures did not show C_2H_2 reduction. The non-graminaceous seed enrichment cultures did not reduce acetylene but upon subculturing in a semi-solid malate medium a diffused bacterial growth consisting mainly of small cocci was observed.

Azospirillum could be isolated from the root pieces of aseptically grown seedlings suggesting that the bacteria present in the seeds colonized the roots. This appears to be a natural mechanism for the continuity of the association. This confirms the results of earlier workers[10,11] who reported the presence of nitrogen-fixing bacteria in the seed.

It is difficult to speculate the source of the organism in the seeds, because it could be isolated from seeds obtained from plants previously inoculated as well as uninoculated. Freshly collected seeds as well as those stored for more than one year harboured this organism. However it is relevant to note here that plant pathogenic bacteria present on the seeds during sowing were reported to enter the roots, move upwards in the vascular bundles and ultimately colonize the seeds[12]. *Azospirillum* has also been observed inside the root tissue including vascular bundles and even in the stem portion[13]. Hence, it may be suggested that the continuity of the association between this organism and graminaceous plants is maintained by a process of cyclic infection from seed-plant-seed. Further the occurrence of *Azospirillum* only in the seeds of graminaceous plants and not others, might be one of the main reasons for the close association of this bacterium with graminaceous plants.

The authors thank A. N. Lahiri for guidance and encouragement.

2 May 1987; Revised 25 August 1987

1. Dobereiner, J. and Day, J. M., In: *First international symposium on nitrogen fixation*, Washington State Univ. Press, Pullman, USA, 1976, p. 518.
2. Subba Rao, N. S., Tilak, K. V. B. R., Singh, C. S. and Lakshmi Kumari M., *Curr. Sci.*, 1979, **48**, 133.
3. Cohen, E., Okon Y., Kigel, J., Nur, I. and Henis, Y., *Plant. Physiol.*, 1980, **66**, 746.
4. Rao, A. V. and Venkateswarlu, B., *Indian. J. Exp. Biol.*, 1982, **20**, 316.
5. Venkateswarlu, B. and Rao, A. V., *Plant Soil*, 1983, **74**, 379.
6. Rai, S. N. and Gaur, A. C., *Plant Soil*, 1982, **69**, 233.
7. Kavimandan, S. K., Subba Rao, N. S. and Mohrir, A. V., *Curr. Sci.*, 1978, **47**, 96.
8. Day, J. M. and Dobereiner, J., *Soil Biol. Biochem.*, 1976, **8**, 45.
9. Tarrand, J. J., Krieg, N. R. and Dobereiner, J., *Can J. Microbiol.*, 1978, **24**, 967.
10. Pohlman, A. A. and Mc Coll, J. G., *Plant Soil.*, 1982, **69**, 341.
11. Ruschel, A. P. and Da Silva W. J., *Associative N_2– fixation*, Vol. 1, CRC Press, Florida, 1981, p. 185.
12. Neergard, P., *Seed pathology*, Macmillan, London, Vol. 1, 1977.
13. Magalhaes, F. M. M., Patriquin, D. and Dobereiner, J., *Rev. Bras. Biol.*, 1979, **39**, 587.