

PEG = Polyethylene glycol

Figure 3. Changes in stomatal resistance in control and PEG (25% w/v in Hoagland solution) treated sunflower seedlings with time.

precedes changes in water potential or stomatal resistance. Such a value of the rate of action potential movement is quite encouraging since Bose⁹ obtained these values falling in the range of 4 to 14 mm s⁻¹ for *Mimosa* in response to mechanical stimuli. In spite of interesting results, Bose's work on electrophysiology did not receive due attention.

An early decrease and further increase in stomatal resistance could be due to a change in membrane permeability caused by electric potential¹⁰. We have also found that the stomatal resistance increases by flowing external electric field through the leaves (data not shown). The depolarization of the membrane causes stomatal closure¹¹.

These events, i.e. changes in electrical potential and stomatal resistance, occur sequentially and in a time scale of seconds to minutes. The transmission speed of electric potential is quite high compared to the transmission speed of a chemical in the phloem¹² or that of ABA, either in the phloem or xylem¹³. More importantly, the stomata closed immediately after the arrival of the action potential¹³. We, therefore, conclude that earliest signal arising from roots under osmotic stress is action potential and which in turn closes stomata. In fact, earlier reports have also shown that the action potential can propagate through plasmodesmata and is capable of triggering a number of physiological and biochemical responses^{14, 15}. In field-grown plants of wheat with and without irrigation, significant differences in resting potential were recorded (data not shown) indicating the possibility of occurrence of this phenomenon under natural environment.

4. Passioura, J. B. and Munns, R., *Aust J Plant Physiol*, 1984, 11, 341-350.
5. Jackson, M. B., in *Communication Between the Roots and Shoots of Flooded Plants* (eds Davies, W. J. and Jeffcoat, B.), Br. Soc. Plant Growth Regul., Bristol, 1990, vol. 21, pp. 115-134.
6. Schurr, U. and Gollan, T., in *Composition of Xylem Sap of Plants Experiencing Root Water Stress - A Descriptive Study* (eds Davies, W. J. and Jeffcoat, B.), Br. Soc. Plant Growth Regul., Bristol, 1990, vol. 21, pp. 201-214.
7. Munns, R. and King, R. W., *Plant Physiol.*, 1988, 88, 703-708.
8. Malone, M. and Stankovic, B., *Plant Cell Environ.*, 1991, 14, 431-436.
9. Bose, J. C., *Philos. Trans. R. Soc. London*, 1913, B204, 63-97.
10. Ullrich, C. I. and Novacky, A. J., *Plant Physiol.*, 1991, 95, 675-681.
11. Kearns, E. V. and Assmann, S. M., *Plant Physiol.*, 1993, 102, 711-715.
12. Wildon, D. C., Thain, J. F., Minchin, P. E. H., Gupp, I. R., Reilly, A. J., Skipper, Y. D., Doherty, H. M., O'Donnell, P. J. and Bowles, D. J., *Nature*, 1992, 360, 62-65.
13. Fromm, J., *Physiol. Plant*, 1991, 83, 529-533.
14. Davies, E., *Plant Cell Environ.*, 1987, 10, 623-631.
15. Robards, A. W. and Lucas, W. J., *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1990, 41, 369-419.

Received 23 March 1994, accepted 9 May 1994

Methane budget from paddy fields in India

D. C. Parashar^{a*}, A. P. Mitra^a, Prabhat K. Gupta^a, J. Rai^a, R. C. Sharma^a, N. Singh^a, S. Kaul^a, G. Lal^b, A. Chaudhary^b, H. S. Ray^c, S. N. Das^c, K. M. Parida^c, S. B. Rao^c, S. P. Kanungo^c, T. Ramasami^d, B. U. Nair^d, M. Swamy^d, G. Singh^e, S. K. Gupta^e, A. R. Singh^e, B. K. Saikia^f, A. K. S. Barua^f, M. G. Pathak^f, C. S. P. Iyer^g, M. Gopalakrishnan^g, P. V. Sane^h, S. N. Singh^h, R. Banerjee^h, N. Sethunathanⁱ, T. K. Adhyaⁱ, V. R. Raoⁱ, P. Palit^j, A. K. Saha^k, N. N. Purkait^k, G. S. Chaturvedi^l, S. P. Sen^m, M. Sen^m, B. Sarkar^m, A. Banik^m, B. H. Subbarayaⁿ, S. Lalⁿ, S. Venkatramaniⁿ and S. K. Sinha^b

^aNational Physical Laboratory, Dr. K. S. Krishnan Road, New Delhi 110 012, India

^bIndian Agricultural Research Institute, New Delhi 110 012, India

^cRegional Research Laboratory, Bhubaneswar 751 013, India

^dCentral Leather Research Institute, Adyar, Madras 600 020, India

^eCentral Fuel Research Institute, Dhanbad 828 108, India

^fRegional Research Laboratory, Jorhat 785 006, India

^gRegional Research Laboratory, Trivandrum 695 019, India

^hNational Botanical Research Institute, Rana Pratap Marg, Lucknow 226 001, India

ⁱCentral Rice Research Institute, Cuttack 753 006, India

^jCentral Research Institute of Jute and Allied Fibres, Barrackpore 743 101, India

^kInstitute of Radio Physics and Electronics, University of Calcutta, 92 Acharya Prafulla Chandra Road, Calcutta 700 009, India

^lNarendra Deo University of Agriculture and Technology, Kumarganj, Faizabad 224 229, India

^mKalyani University, Kalyani 741 235, India

ⁿPhysical Research Laboratory, Navrangpura, Ahmedabad 380 009, India

*For correspondence

1. Bansal, K. C. and Sinha, S. K., *Euphytica*, 1991, 56, 7-14.

2. Gowing, D. J., Davies, W. J. and Jones, H. G., *J. Exp. Bot.*, 1990, 41, 1535-1540.

3. Passioura, J. B., *Aust. J. Plant Physiol.*, 1988, 15, 687-693.

Earlier measurements¹ of methane emission from rice paddy fields yielded a low methane budget of around 3 Tg/yr for India, and was reported at IPCC forums². This low budget was based on limited data. An extensive national methane measurement campaign from Indian paddy fields was launched in 1991, which involved a number of scientific institutions and universities with the National Physical Laboratory (NPL) at Delhi operating as a nodal agency. The results obtained during this campaign based on more than 2000 observations, covering the complete 1991 kharif (monsoon) season are reported here. The methane emission rates ranged between -0.20 and $66 \text{ mg m}^{-2} \text{ hr}^{-1}$. The irrigated fields (intermittently flooded) had the values between -0.20 and $3.6 \text{ mg m}^{-2} \text{ hr}^{-1}$ (seasonal integrated flux 0.05 to 2 g m^{-2}), water logged (flooded) fields between 0.04 to $66 \text{ mg m}^{-2} \text{ hr}^{-1}$ (seasonal integrated flux 10 – 60 g m^{-2}) and deep water regimes in the range between 1.10 and $23.3 \text{ mg m}^{-2} \text{ hr}^{-1}$ (seasonal integrated flux 14 to 24 g m^{-2}). The methane budget from Indian paddies has been estimated to have an average value of 4.3 Tg yr^{-1} and ranges between 2.7 and 6.4 Tg yr^{-1} .

ASIAN region has about 90% of total world rice harvested area [147.52 million hectare (mha)] of which 42.2 mha lies in India³. On the basis of extrapolation of measurements done in Europe and USA, US-EPA attributed $37.8 \text{ Tg CH}_4 \text{ yr}^{-1}$ to Indian rice paddies, which was an order of magnitude more than our earlier estimates¹ for India. To obtain more reliable CH_4 budget estimate from Indian paddy fields, methane campaign in 1991 (MC91) during the whole kharif (June to November) paddy cropping season was organized involving more than 15 organizations.

Static box and gas chromatographic technique¹ was used with 1 ppmv methane in nitrogen obtained from M/s Matheson Gas Products, USA for calibration as primary standard. The accuracy and compatibility of methane concentration values were established internationally. The methane emission rates at different locations were found to vary despite having the same soil, paddy variety, age and water management practices. Therefore the seasonal integrated flux (SIF) of methane and its range were worked out for each network station by taking the daily mean of the flux data and integrating it over the whole cropping season. Extrapolations for the gap periods in the cropping season were made to estimate the total SIF. The data scattered above and below the mean flux were considered to obtain the maximum and minimum ranges of SIF for each station. The SIF and its range so obtained were used in various paddy cultivation water regimes for estimating the methane budget for India.

Total paddy harvested area, including multiple cropping, in different states and union territories of India was classified³⁻⁸ under irrigated, rainfed water-

logged, deep water and upland paddy water regime categories. The average SIF value of various network stations in the different paddy water regimes located in a particular state was applied to estimate the total methane emission for that state. Methane emission from paddy fields under various categories from states/union territories not having any network station was estimated by applying the mean SIF of the stations located around that region on the basis of similar agroclimatic environment particularly rainfall.

Some aspects that need to be emphasized include an order of magnitude higher emissions from rainfed waterlogged than from irrigated paddy fields. The range of SIF from rainfed waterlogged areas was quite large (Table 1) and may be due to varying hydrological conditions from place to place not only in different maximally sustained flooding depths, but in the flooding duration as well. The total area covering the irrigated and upland fields was as large as 53% for India and 66% for the world⁷, and since this large area essentially did not play a significant part in the methane budget, any estimate of total methane emission without considering these facts would be unrealistic. The measurements in irrigated fields without continuous waterlogging were scarce and virtually limited to those by the present authors in India. Experiments in China, USA, Spain, Italy, Japan, Thailand and Philippines were performed mainly in artificially flooded fields and a standing water level of a few centimetres was maintained⁹⁻¹⁹. These, therefore, represented conditions of what we term here as 'rainfed waterlogged' areas and cannot truly represent 'irrigated' areas⁶. This, in fact, was the difference between the inventories by the present authors and those done by others (Figure 1). In Figure 1 the SIF ranges shown for Nanjing and Beijing (China), and Philippines

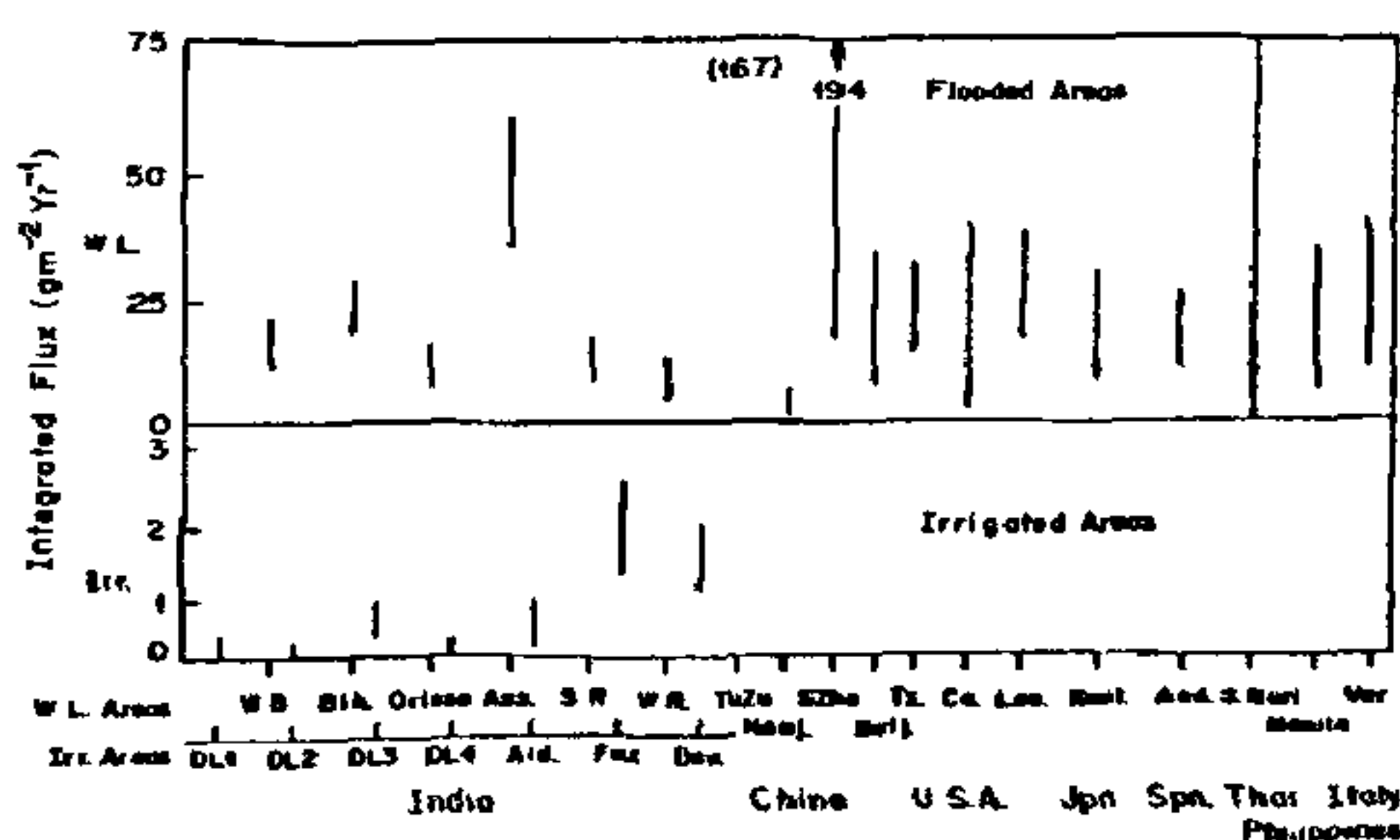


Figure 1. Seasonal integrated emission of methane from rice paddy fields (a comparison). Abbreviations used W.L.-Waterlogged, W.B.-West Bengal; Bih-Bihar, Ass-Assam, S.R.-Southern Region; W.R.-Western Region, Tx-Texas, Ca-California, Lou-Louisiana, Kant-Kantou Plain, And-Andalusia, S.Bur-Suphan Buri; Var-Vercelli, Irr-Irrigated, DL1 to DL4 Delhi Sites 1 to 4, Ald-Allahabad, Faz-Faizabad, Dev-Devoke, Jpn-Japan, Spn-Spain, Thai-Thailand

Table 1. Methane budget estimates from Indian paddy fields

Areas Regions/States	Cultivation area (mha)	Integrated seasonal methane flux (g m ⁻²)			Total methane emission (Tg Yr ⁻¹)		
		Max.	Mean	Min.	Max	Mean	Min.
A Rainfed waterlogged areas							
I Eastern Region							
a) West Bengal	2 457	34 ± 10	28	23 ± 6	0.84 ± 0.25	0.69	0.57 ± 0.15
b) Bihar	2 235	26 ± 2	22	19 ± 4	0.58 ± 0.05	0.49	0.43 ± 0.09
c) Orissa	2 109	14 ± 5	12	10 ± 3	0.30 ± 0.11	0.25	0.21 ± 0.06
d) Assam	1 590	60 ± 2	46	35 ± 3	0.95 ± 0.03	0.73	0.56 ± 0.05
e) N E States	0 219	60 ± 2	46	35 ± 3	0.13 ± 0.004	0.10	0.08 ± 0.007
II Southern Region							
a) Andhra Pradesh	1 245						
b) Tamil Nadu	0 593	13 ± 2	11	9 ± 2	0.26 ± 0.04	0.21	0.17 ± 0.04
c) Pondicherry	0 025						
d) Kerala	0 017	13 ± 1	9	5 ± 1	0.002 ± 0.0002	0.002	0.001 ± 0.0002
e) Andaman & Nicobar Islands	0 012	22 ± 10	17	12 ± 6	0.002 ± 0.001	0.002	0.0014 ± 0.0007
III Northern Region							
Uttar Pradesh	3.174	26 ± 2	22	19 ± 4	0.83 ± 0.06	0.70	0.60 ± 0.13
IV Western Region							
a) Madhya Pradesh							
b) Maharashtra	3 377	16 ± 8	14	11 ± 4	0.54 ± 0.27	0.47	0.37 ± 0.14
c) Gujarat							
d) Goa, Daman & Diu	0.275	13 ± 1	9	5 ± 1	0.036 ± 0.003	0.03	0.014 ± 0.003
e) Dadar Nagar Haveli							
Total	17.328				4.17 ± 0.82	3.67	3.01 ± 0.67
B Deep water areas	2.434	24 ± 2	19	14 ± 1	0.58 ± 0.046	0.46	0.342 ± 0.025
C Irrigated areas	16 497	2 ± 0.6	0.74	0.06 ± 0.01	0.335 ± 0.078	0.122	0.061 ± 0.007
D Upland areas	5.973		Negligible			Negligible	
Grand total	42.232				5.4 ± 1.0	4.3	3.4 ± 0.7

were calculated by multiplying minimum and maximum ranges of methane emission per day by 30 days²⁰. The difference was not in the flux rates between those obtained by us and by others but in the application of these fluxes to the appropriate area for the various paddy water regimes. The other aspect contributing to the varying budget estimate concerned the choice of the number of vegetation days from 120 to 150 considered by others⁹⁻¹¹ in calculation of budget estimates. In India the vegetation days ranged from 73 to 130 days of which rainfed flooding days would be fewer resulting in less emission days for the methane flux from paddy fields. We had, therefore, concentrated on integrated flux rates (SIF) rather than on daily mean flux rate multiplied by number of vegetation days.

With these considerations, the methane budget was worked out for different areas as given in Table 1. It was found that 94% of total emission was from rainfed waterlogged category and 6% of the emission from irrigated paddy water regimes. The methane emission

from Indian paddy fields came out to be between 2.7 and 6.4 Tg yr^{-1} with a mean of 4.3 Tg yr^{-1} .

1. Parashar, D. C., Rai, J., Gupta, Prabhat K. and Singh, N., *Indian J. Radio Space Phys.*, 1991, 20, 12-17.
2. Houghton, J. T., Callander, B. A. and Varney, S. K., in *Climate Change 1992*, Supplementary Report to the IPCC Scientific Assessment, Cambridge University Press, 1992, pp. 35-37.
3. Estimation of green house gases and sinks, in OECD/OCDE final report for IPCC, Environment Directorate, Paris, 1991, pp. 5-43.
4. Tunwar, N. S. and Singh, S. V., in *Handbook on Cultivars*, Central Seed Committee Publication, Department of Agricultural Cooperation, Min. of Agr. & Rural Development, New Delhi, 1985, pp. 8-11.
5. Gupta, P. C. and Toole, J. C. O., in *Upland Rice: A Global Perspective*, IRRI, Philippines, 1986, pp. 1-5.
6. *Terminology for Rice Growing Environments*, IRRI, Los Banos, Laguna, Manila, Philippines, 1984, pp. 5-24.
7. *Progress in Rainfed Lowland Rice*, IRRI, Los Banos, Philippines, 1986, pp. 4-399.
8. *All India Final Estimates of Rice: 1989-1990*, Ministry of Agriculture, Government of India, New Delhi, 1990.

- 9 Khalil, M. A. K., Rasmussen, R. A., Wang, M.-X. and Ren, L., *Environ Sci Technol.*, 1991, 25, 979-981.
- 10 Wang, M.-X., Dai, A. G., Huang, J., Ren, L., Seiler, W., Schutz, H., Rennenberg, H., Rasmussen, R. A. and Khalil, M. A. K., in *Proceedings of CH₄ and N₂O workshop*, NIAES, Tsukuba, Japan, March 25-26, 1992, pp. 70-88.
- 11 Wang, M.-X., Aiguo, D., Renxing, S., Schutz, H., Rennenberg, H., Seiler, W. and Haibao, W., *Acta Meteorol Sin.*, 1989, 4, 265-275.
- 12 Cicerone, R. J. and Shetter, J. D., *J. Geophys. Res.*, 1981, 86, 7203-7209.
- 13 Cicerone, R. J., Shetter, J. D. and Delwiche, C. C., *J. Geophys. Res.*, 1983, 88, 11022-11024.
- 14 Sass, R. L., Fisher, F. M., Harcombe, P. A. and Turner, F. T., *Global Biogeochemical Cycles*, 1990, 4, 47-68.
- 15 Seiler, W., Holzapfel-Pschorn, A., Conrad, R. and Scharffe, D., *J. Atmos. Chem.*, 1984, 1, 241-268.
- 16 Schutz, H., Holzapfel-Pschorn, A., Conrad, R., Rennenberg, H. and Seiler, W., *J. Geophys. Res.*, 1989, 94, 16405-16416.
- 17 Yagi, K. and Minami, K., *Soil Science Plant Nutr.*, 1990, 36, 599-610.
- 18 Yagi, K. and Minami, K., in *Proceedings of CH₄ and N₂O workshop*, NIAES, Tsukuba, Japan, March 25-26, 1992, pp. 89-105.
- 19 Neue, H. U., Lantin, R. S., Alberto, M. C. R., Aduna, J. B., Flores, M. J. and Tan, M. J., in *Proceedings of CH₄ and N₂O workshop*, NIAES, Tsukuba, Japan, March 25-26, 1992, pp. 43-45.
- 20 Mitra, A. P. (ed.), in *Global Change: Greenhouse Gas Emissions in India—A Preliminary Report No. 1*, PID, CSIR New Delhi, 1991, pp. 18-22.

ACKNOWLEDGEMENTS. We are grateful to Dr E. S. R. Gopal, Director NPL and Dr K. Lal, Head, Material Characterization Division, NPL, for their keen interest in this work. The financial support from the Ministry of Environment and Forests, Government of India and technical help from the National Council of Science Museums, Calcutta is gratefully acknowledged. Thanks are due to Dr S. C. Majumdar, Dy. Director, CSIR, New Delhi for help throughout this work. We thank the vice-chancellors of agricultural universities and directors of ICAR institutes who helped in organizing the experiments. Kind help for intercomparison and calibration from Dr P. J. Crutzen, Max Planck Institute of Chemistry, Mainz, Germany, Dr K. Minami, NIAES, Tsukuba, Japan and Dr P. Fraser and Dr P. Steele, CSIRO, Australia is gratefully acknowledged.

Received 16 March 1994; accepted 24 March 1994

***In vitro* seed germination and plantlet establishment of two wild relatives of sweet potato (*Ipomoea trifida* and *I. amnicola*)**

Archana Mukherjee and B. Vimala

Central Tuber Crops Research Institute,
Thiruvananthapuram 695 017, India

Seeds of two wild relatives of sweet potato, *Ipomoea trifida* and *I. amnicola*, introduced from Peru

germinated only *in vitro* in MS medium. Whereas the growth and establishment of plantlets of *I. amnicola* could be achieved on MS itself, *I. trifida* required 1-naphthaleneacetic acid (NAA) in the medium. *In vitro* derived plantlets were successfully established in soil in pots.

UTILIZATION of wild species for improvement of sweet potato has been well explained¹. Development of nematode-resistant varieties of sweet potato 'Minamiyutaka'² and 'Okuyutaka'³ using the wild relative (*Ipomoea trifida* or K 123-11) has further proved the potential of the wild relatives in sweet potato breeding. *I. trifida*, which occurs as different cytotypes, is considered most closely related to sweet potato. Use of these cytotypes in sweet potato breeding by ploidy level manipulation has been reported^{4,5}. Introduction and establishment of the wild relatives therefore assume importance. We report the results of our studies on seed germination and establishment of two wild species, *I. trifida* and *I. amnicola*.

Ten seeds of each species obtained from the International Potato Center (CIP), Lima, Peru, and kept stored at -20°C for 48 weeks were treated with concentrated H₂SO₄ for 1 h. After thorough washing in tap water six seeds of each species were transferred onto moist blotting paper in petri dishes and maintained at 28 ± 2°C. The remaining four seeds were surface-sterilized in 0.1% mercuric chloride for 7 min and cultured on Murashige and Skoog (MS) medium⁶ containing B-vitamins and 3% sucrose. The cultures were maintained at 25 ± 2°C under 8 h photoperiod (irradiance 30 µE m⁻² s⁻¹). The resulting plantlets were transplanted into soil + sand mixture (1:1) in pots and were maintained for 4-5 weeks under diffuse sunlight, 28 ± 2°C, 70-75% RH, and were subsequently transferred to open field and watered periodically.

None of the seeds germinated on the moist blotting paper in the petri dishes; on MS two of the four seeds of each species germinated within a week. *In vitro* seed germination of *I. amnicola* was similar to that reported for sweet potato⁷. Shoot development was faster in *I. amnicola* than in *I. trifida*. The primary shoots which developed in 4 weeks from seed culture were multiplied by single node cuttings (4-5 mm long) and transferred to MS control medium and MS + NAA (1 µM and 2 µM). *I. trifida* needed NAA in the medium for root production, whereas *I. amnicola* rooted on MS itself (Table 1). On MS, axillary shoots developed in *I. amnicola* only. In *I. trifida*, although axillary shoots developed after transfer to fresh MS, they senesced. Transfer of such cultures to MS + NAA resulted in root formation as well as further development of the axillary shoots. Thus, *I. trifida* cultures needed NAA for formation of plantlets. Of the two concentrations of NAA tested, vigorous plantlet growth occurred on