Table 1 *Effect of oxyfluorfen on PS II activity of normal and heat-inactivated spinach chloroplasts*

Treatment	PS II activity* (μmol DCIP reduced/mg chl/h) Control	Heat-treated
—	380 ± 67	48 ± 2
Oxyfluorfen	8 ± 1.2	0
Oxyfluorfen + DPC	4 ± 0.6	0
DPC	—	172 ± 3

*Each value is mean ± SD of triplicate samples. The data are representative as rate of DCIP reduction in control chloroplasts varied from 350 to 400 μmol DCIP reduced/mg chl/h in different preparations. Other conditions are as in text.

electron-accepting site on the oxidizing side of PS II. Increase in I_{50} with increase in chloroplast concentration suggests that oxyfluorfen may bind to pigment complex(es) or some other membrane component. The binding of oxyfluorfen affects PS II photochemistry[18].

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1. Yin, R. Y., *J. Agric. Food Chem.*, 1975, **23**, 592.
2. Gorske, S. F. and Hopen, H. J., *Weed Sci.*, 1978, **26**, 585.
3. Matsunaka, *J. Agric. Food Chem.*, 1969, **17**, 171.
4. Fadayomi, O. and Warren, G. F., *Weed Sci.*, 1976, **24**, 598.
5. Vanstone, D. E. and Stobbe, E. H., *Weed Sci.*, 1977, **25**, 352.
6. Boger, P., *Z. Naturforsch.*, 1984, **39C**, 468.
7. Alscher, A. and Strick, C., *Pest. Biochem. Physiol.*, 1984, **21**, 248.
8. Gillman, D. J., Evans, M. W. and Dodge, A. D., *Proc. Br. Crop. Protect. Conf. Weeds*, 1985, **7**, 1195.
9. Duke, S. D. and Kenyon, W. H., *Z. Naturforsch.*, 1987, **42C**, 813.
10. Ensminger, M. P. and Hess, F. D., *Plant Physiol.*, 1985, **78**, 46.
11. Haworth, P. and Hess, F. D., *Plant Physiol.*, 1988, **86**, 72.
12. Hess, F. D. and Orr, G. L., *Abstr. Weed Soc. Am. Meet., nos.*, 1981, p. 246.
13. Kunert, K. J. and Boger, P., *Weed Sci.*, 1981, **29**, 169.
14. Karabourniotis, G., Menetas, Y. and Gavrees, N. A., *Plant Physiol.*, 1985, **77**, 300.
15. Hall, D. O., *Nature (London)*, 1972, **235**, 125.
16. Mackenny, G., *J. Biol. Chem.*, 1941, **140**, 315.
17. Mohanty, P., Papageorgiou, G. and Govindjee, *Photochem. Photobiol.*, 1971, **14**, 667.
18. Sharma, D., Bhardwaj, R., Maheshwari, V. and Nagar, S., *Plant Sci.*, 1989, **63**, 1.

ELECTRICAL CONDUCTIVITY AS AN INDICATOR OF ORTHODOX AND RECALCITRANT SEED PHYSIOLOGY IN TROPICAL FOREST TREES

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THE majority of seeds used in agriculture show orthodox behaviour, that is to say, over a wide range of environmental conditions, their longevity may be increased in specific and predictable ways by decreasing temperature and moisture content. Recalcitrant seeds are those that do not obey these rules since they are killed when their moisture content is reduced below some relatively low value[1]. Such seeds cannot be preserved for more than a few months and some can only survive for a few weeks even when desiccation is prevented[2,3]. Diagnosis of the recalcitrant condition is not always straightforward, and there are many cases of seeds initially classified as recalcitrant and now known to be typically orthodox[4]. In the case of tropical forests where a number of forest tree species are recalcitrant, a quick and reliable method for identification of such species is absent.

Incorrect diagnosis of orthodox and recalcitrant seeds can affect decisions on methods of genetic conservation. The present study is the first attempt to find a measure for the identification of these two seed categories, taking seeds from truly recalcitrant and orthodox species. Experiments were conducted on freshly collected mature seeds of three truly recalcitrant species, viz. *Shorea robusta* Gaertn f., *Eugenia jambolana* Lam. and *Saraca indica* Linn., and four orthodox species, viz. *Bauhinia variegata* Linn., *Bombax malabarcium* D. C., *Dalbergia sissoo* Roxb. and *Melia azadirachta* Linn. Germination of seeds in the seven species was found to be 80 to 100%. Electrical conductivity was determined[5] hourly up to 24 h after immersing seeds in water at 20°C.

Figure 1 shows that electrical conductivity in recalcitrant seeds ranged from 1.36 to 14.72 $\mu s\,cm^{-1}\,g^{-1}$.

In the case of orthodox seeds the range was 27.84 to 171.56 $\mu s\ cm^{-1}\ g^{-1}$ (figure 2). Since electrolyte leakage is an indicator of membrane disruption,[6] the results of the present study suggest that, in orthodox seeds, cell membranes lose their integrity because of drying to a greater extent on the tree itself[7,8]. The

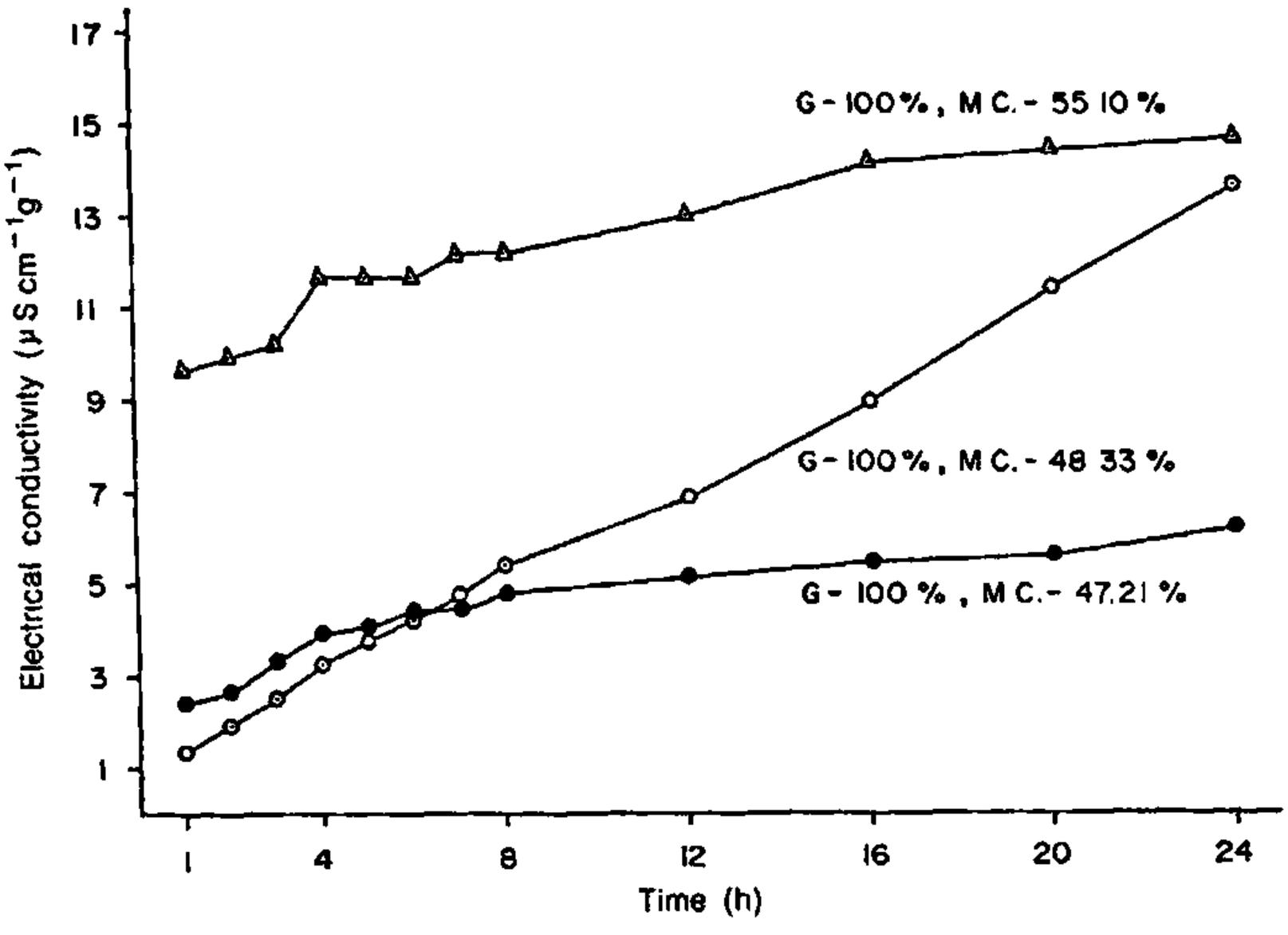

Figure 1. Time course of electrolyte leakage from recalcitrant seeds of *S. robusta* (○), *S. indica* (●), and *E. jambolana* (△) immersed in water (G, germination; MC, moisture content).

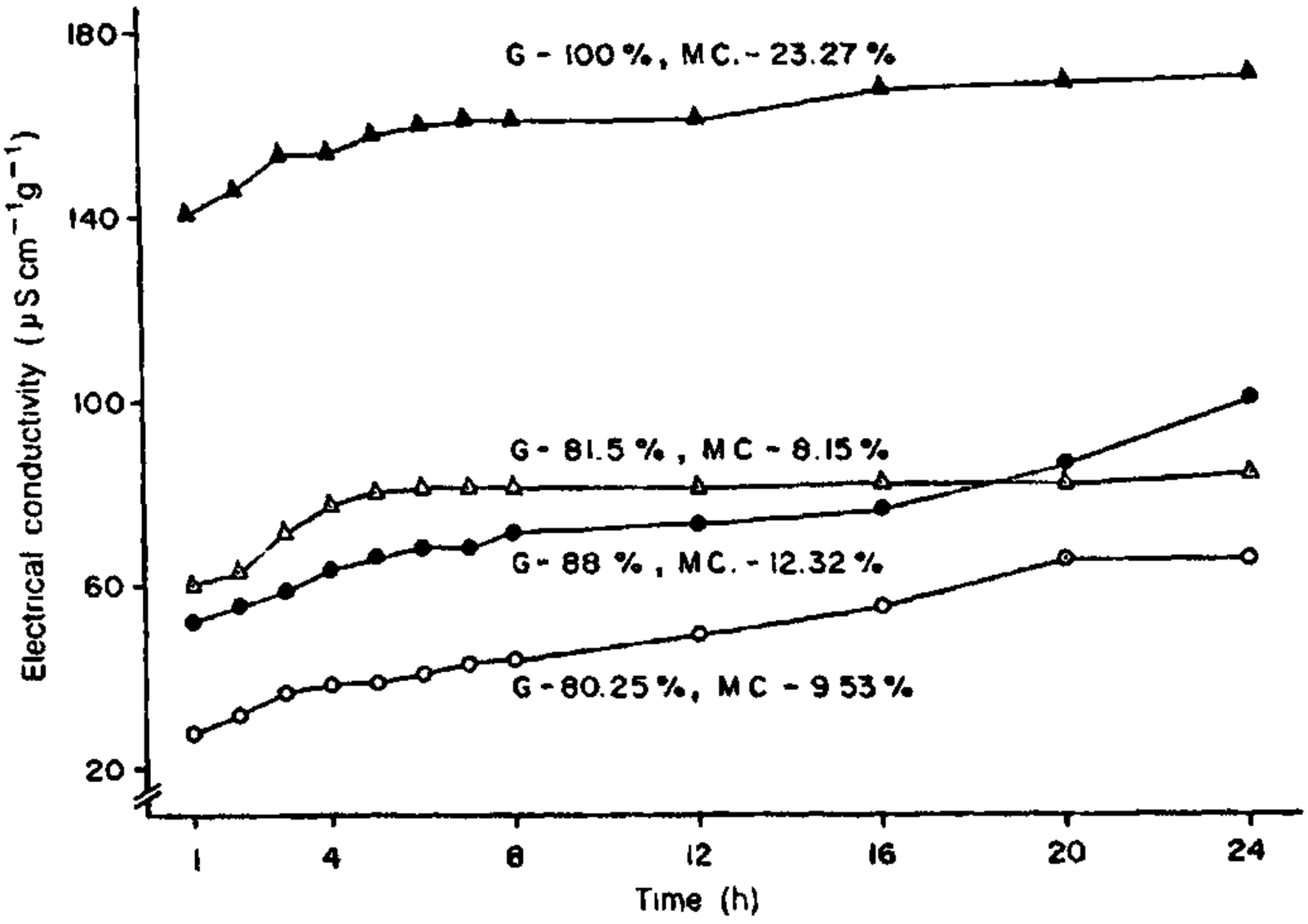

Figure 2. Time course of electrolyte leakage from orthodox seeds of *B. variegata* (○), *D. sissoo* (●), *B. malabaricum* (△), and *M. azadirachta* (▲) immersed in water (G, germination; MC, moisture content).

results can also be interpreted on the basis of low moisture content in the orthodox seeds, except *M. azadirachta*, where seed moisture content was comparatively higher (23.27%) owing to the fleshy outer covering, which delayed the process of seed desiccation on the tree itself. The higher seed moisture content in this species can also be explained on the basis that mature seeds were considered for the experiment without manipulating seed moisture. However, 20% germination has been reported in this species for seeds with 4% moisture content[4]. The high moisture content (48.33 to 55.10%) in recalcitrant seeds indicates that, because of slow desiccation rate, membrane integrity is maintained and leakage is less. This situation may have evolved because orthodox species benefit from desiccation while slow desiccation rate is protective in recalcitrant species[9]. In contrast to the higher electrolyte leakage from orthodox seeds and less leakage from recalcitrant seeds, the pattern of rate of loss of electrolyte was similar in both the categories (tables 1 and 2). Maximum electrolyte leakage was observed during the first hour of soaking, followed by a decline. This decline and/or stabilization in the rate of loss can be attributed to the re-establishment of membrane integrity in the seeds due to a repair mechanism on hydration.

Among the seven species in the present study, *M. azadirachta* was earlier recognized as recalcitrant[10] but later included in the orthodox category owing to the presence of structural features such as a fleshy outer covering on a relatively thin-walled stone which contains the kernel. However, if kernels are removed from dried stones before the germination test, seeds germinate perfectly satisfactorily. It is not clear in this case whether the stones prevent germination entirely or delay it considerably[4]. Higher electrolyte leakage observed in this study from seeds of this species also confirms their orthodox nature.

Electrical conductivity of seeds could be used as a measure for identification of orthodox ($>20\ \mu S\ cm^{-1}\ g^{-1}$) and recalcitrant seeds ($<20\ \mu S\ cm^{-1} g^{-1}$). It is further suggested that conductivity measurements should be performed on freshly collected seeds to avoid desiccation effects during storage.

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Table 1 *Rate of loss of electrolytes from recalcitrant seeds of three forest tree species after different soaking periods*

Soaking period (h)	Electrolyte loss rate (%)		
	S. robusta	*S. indica*	*E. jambolana*
1	9.96	37.67	65.37
2	4.24	4.46	1.95
3	4.49	10.01	1.90
4	4.98	10.81	9.65
5	3.94	2.23	0
6	3.46	4.29	0
7	3.88	2.23	3.80
8	4.76	4.29	0.0
12	18.17	6.51	5.78
16	7.91	4.46	8.74
20	18.3	2.07	1.90
24	15.83	9.97	1.91

Table 2 *Rate of loss of electrolytes from orthodox seeds of four forest tree species after different soaking periods*

Soaking period (h)	Electrolyte loss rate (%)			
	A. indica	*D. sissoo*	*B. variegata*	*S. malabaricum*
1	82.19	51.60	42.29	70.93
2	3.42	3.23	5.77	3.42
3	4.10	3.22	7.68	10.25
4	0	4.83	1.92	6.84
5	2.73	3.22	0	3.42
6	0.68	1.61	3.84	0.84
7	0.68	0	3.84	0
8	0	3.22	1.92	0
12	0	1.61	7.68	0
16	4.10	3.23	9.61	0.86
20	0.68	9.67	15.39	0
24	1.36	14.51	0	3.42

1. Roberts, E. H., *Seed Sci. Technol.*, 1973, **1**, 499.
2. King, M. W. and Roberts, E. H., *The Storage of Recalcitrant Seeds: Achievements and Possible Approaches*, IBPGR, 1979.
3. King, M. W. and Roberts, E. H., In: *Recalcitrant Crop Seeds*, (eds) H. F. Chin and E. H. Roberts, Tropical Press, Kuala Lumpur, 1980, p. 153.
4. Roberts, E. H., King, M. W. and Ellis, R. H., In: *Crop Genetic Resources: Conservation and Evaluation*, (eds) J. H. W. Holden and J. T. Williams, George Allen and Unwin, London, 1984, p. 38.
5. Matthews, S. and Powell, A., *Handbook of Vigour Test Methods*, ISTA, Switzerland, 1981.
6. Roberts, E. H. and Ellis, R. H., In: *The Physiology and Biochemistry of Germination*, (ed.)

A. A. Khan, Elsevier Biomedical Press, Amsterdam, 1982, p. 465.
7. Simon, E. W. and Raja Harum, R. M., *J. Exp. Bot.*, 1972, **23**, 1076.
8. Simon, E. W., *New Phytol.*, 1974, **73**, 377.
9. Tompsett, P. B., *Proc. Int. Symp. on Forest Seed Problems in Africa*, (eds) S. K. Kamra and R. D. Ayling, IUFRO, 1987, p. 348.
10. Roberts, E. H., *Adv. Res. Technol. Seeds*, 1983, **8**,

PHYSIOLOGY OF MUTANT STRAINS OF *ERWINIA CAROTOVORA* PRODUCING L-ASPARAGINASE

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SINCE the first demonstration[1] of L-asparaginase (L-asparagine amidohydrolase EC 3.5.1.1) as an anticancer enzyme, production of the enzyme by a variety of microbes has been investigated in many laboratories. The various sources reported, such as *Pseudomonas fluorescens*[2], *Serratia marcescens*[3], *Escherichia coli*[4], *Erwinia carotovora*[5] and *Proteus vulgaris*[6], provided active and immunologically different enzyme preparations. All these species were evaluated in the author's laboratory. As *E. carotovora* was shown to be a promising culture, it was mutated by UV and gamma radiation and by treatment with chemical mutagens as part of a strain improvement programme. The mutant strains derived by these traditional methods were further investigated by a classical method of genetic recombination reported by Barnes *et al.*[7]

The original wild-type strain *E. carotovora* was a gift from Prof. Wade of Microbiological Research Establishment (UK). It was mutated by the routine methods such as UV and gamma radiation and treatment with chemical mutagens. The mutants were screened and stock cultures were maintained. Working cultures were obtained by streaking the stock culture and transferring isolated colonies on tryptone yeast (TY) agar slants (in g/l: Tryptone, 5.0; yeast extract, 2.0; lactose, 5.0; K_2HPO_4, 1.0; NaCl, 5.0; agar, 15.0; pH 6.8–7.0). Purity was checked periodically.

Auxotrophic strains were isolated on minimal agar (in g/l: NaCl, 0.2; Na_2SO_4, 2.0; K_2HPO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.2; lactose, 10; pH 7.0) supplemented with individual amino acids. To determine antibiotic resistance, the following antibiotics were added to minimal agar: penicillin, 200–2000 units/ml; ampicillin, 10–20 μg/ml; erythromycin, 5–10 μg/ml; chlortetracycline, 10–100 μg/ml; chloramphenicol, 250–500 μg/ml; and streptomycin, 10–20 μg/ml.

Complete medium (TGY) was used for the fermentation process (in g/l: Tryptone, 20.0; yeast exrtact, 5.0; monosodium glutamate, 15.0; lactose, 10.0; K_2HPO_4, 1.0; NaCl, 1.0; $CaCO_3$, 5.0; $MgSO_4 \cdot 7H_2O$, 0.2; pH 7.4).

The depleted mutant strains of *E. carotovora* were treated with a mixture of amino acids which included monosodium glutamate, L-asparagine, tryptophan, methionine, alanine, serine and threonine. After eliminating one of these amino acids from the mixture, their polyauxotrophic character was determined. They were screened to select producer and non-producer strains and tested for growth in presence of antibiotics. The producer strains were resistant particularly to streptomycin and chlortetracycline antibiotics at the higher concentration. They required specifically monosodium glutamate as carbon source. The non-producer strains lacked this requirement and their growth was inhibited at lower concentrations of streptomycin and chlortetracycline. All the strains—wild and polyauxotrophic—were however sensitive to penicillin and ampicillin.

The mutants were mated according to the procedure of Barnes *et al.*[7] Donor, recipient and recombinant cells were characterized by observing their growth on the third day. Only genetic recombinants grew and were picked up as isolated colonies. They were subcultured five times on minimal agar plates and stock cultures were prepared. After purification they were transferred to agar slants, incubated for 16 h, and inoculated into production flasks directly for fermentation studies. The fermentation was carried out in 100 ml medium in a 500 ml Erlenmeyer flask. The cultures were incubated for 24 h at 37°C on a rotary shaker (250 rpm). Changes in fermentation parameters in the recombinants were considered as evidence of successful application of the technique adopted. L-Asparaginase activity, being intracellular, was assayed by the routine Nesslerization method[8]. One IU represents 1 μmol of ammonia liberated per min at 37°C in 0.05 M borate buffer, pH 8.5.

In table 1, the polyauxotrophic and the streptomycin-sensitive (Sm^S) strains are shown as non-producers while the streptomycin-resistant (Sm^R)