lethal test in mice[5,6]. A tendency toward an increased frequency of chromosome aberrations after arsenic in vivo treatment was found in marrow cells of Chinese hamsters[5]. Considering these contradictory data, an in vivo test was performed to estimate the mutagenicity of arsenic in bone marrow cells and in spermatogonial cells of mice.

Material and methods. Arsenic, as As_2O_3 compound was dissolved in distilled water by adding NAOH and this solution was neutralized. Male Swiss Albino OF1 mice, 12 weeks old, were given a single i.p. injection of 0.5 ml of the arsenic solution with a concentration of 0; 4; 8 and 12 mg As/kg b.wt. Control animals were injected with 0.5 ml distilled water. Animals were sacrificed after recovery periods of 12, 24, 36 and 48 h. Microscopic slides were prepared using the 'blowing' technique[7,8]. Cytogenetic damage was evaluated by determining the frequencies of gaps, breaks and exchanges in bone marrow cells and in spermatogonia.

Results and discussion. In the bone marrow cells, no dose-effect relationship was found either for gaps, or for chromatid and for chromosome type aberrations (table 1). No significantly increased frequency of breaks and exchanges was observed. However, a concentration of 4 mg As/kg b.wt and a concentration of 8 mg As/kg b.wt induce significantly more gaps in animals killed after 48 h ($p < 0.01$) and 36 h ($p < 0.05$) respectively than in nontreated mice.

Since gaps are rather unreliable measures, it may be considered that arsenic does not induce chromatid or chromosome aberrations in bone marrow cells. The spermatogonial cells also show no significant increase of gaps, chromatid or chromosome type aberrations after arsenic administration (table 2).

Our data confirm that parameters of mutagenicity such as chromatid and chromosome aberrations, are not increased by arsenic in vivo in mammals. This does not exclude mutagenic action of arsenic through other mechanisms, such as repair inhibition, DNA polymerase inhibition.

Taking into account the hypothesis of an inhibiting effect of arsenic on the repair mechanism, a combined exposure to arsenic and a known mutagenic agent may help to elucidate the action of arsenic in vivo.

1 Acknowledgments. We thank Mr L. De Langhe for skillfull technical help. We acknowledge this work was supported by a grant of the Ministery of Public Health.

2 P. Ehrlich, Ber. dt. chem. Ges. *42*, 17 (1909); L.S. Goodman and A. Gilman, The pharmacologual basis of therapeutics, 3rd edn. Macmillan, New York 1965.
3 J. Petres, D. Baron and I. Kunick, Derm. Mschr. *160*, 724 (1974).
4 A. Leonard, and R. Lauwerys, Mutation Res. *75*, 49 (1980).
5 G. Dugatóvá, Acta Fac. Rerum nat. Univ. comen., Bratisl. *9*, 63 (1978), and references cited therein.
6 R.J. Šrám and V. Bencko, Czech, Hyg. *19*, 308 (1974).
7 Evans, Cytogenetics *3*, 289 (1964).
8 A. Leonard and G. Deknudt, Acta zool. path. Antverp. *48*, 43 (1969).

Isocitrate dehydrogenase gene duplication and fixed heterophenotype in the cultivated soybean *Glycine max*

H.S. Yong, K.L. Chan, C. Mak and S.S. Dhaliwal

Department of Genetics and Cellular Biology, University of Malaya, Kuala Lumpur (Malaysia), 21 April 1980

Summary. Isocitrate dehydrogenase (E.C. 1.1.1.42) gene duplication was demonstrated in the self-pollinated soybean *(Glycine max)* by means of starch-gel electrophoresis. This finding explains the heterogeneity and/or fixed heterophenotype observed in some soybean cultivars.

The cultivated soybean *(Glycine max)* is normally self-pollinated; natural cross-pollination is usually considerably less than 1%. Like other self-pollinated plants, cultivars (or varieties) of soybean are expected to have attained homozygosity in their genetic constitution. This appears to be so for seed proteins, INT-oxidase, peroxidase, urease, esterase activity, acid phosphatase, alcohol dehydrogenase, amylase and tetrazolium oxidase, which have been demonstrated to be varietyspecific[1-6]. In our survey for inter-varietal differences employing biochemical characters, a number of soybean cultivars of diploid origin were found to be heterogeneous with respect to isocitrate dehydrogenase (E.C. 1.1.1.42). This is rather unexpected and we report here our finding of isocitrate dehydrogenase gene duplication and fixed heterophenotype in the cultivated soybean.

Conventional horizontal starch-gel electrophoresis employing the citrate-phosphate buffer system at pH 6.8 reveals that isocitrate dehydrogenase in soybean is represented by at least 4 electrophoretic patterns at the faster anodal zone (figure). There is also a distinctly slower anodal zone *(Idh-S)* which is represented by a single band and appears to be variety-specific.

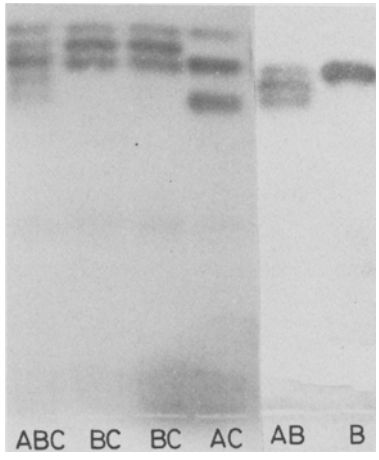
The electrophoretic patterns observed in 12 varieties of soybean are summarized in the table. Of these varieties, 7 are homogeneous and 5 heterogeneous. The homogeneous varieties are of 3 distinct types (IDH-B, IDH-AB and IDH-BC respectively), while the heterogeneous varieties fall into 2 groups (with IDH-B and IDH-AB or IDH-BC and IDH-AC respectively).

Electrophoretic studies of seeds produced by individual plants reveal that their IDH-phenotype is the same, and is identical to that of the parental plant; e.g., an IDH-AC plant produces only seeds with the IDH-AC phenotype. On

Distribution of IDH-F electrophoretic patterns in 12 varieties of cultivated soybean

Variety	IDH-F phenotypes			
	B	AB	BC	AC
BM 10	39	–	–	–
Clark 63	51	–	–	–
KS-437	–	31	–	–
Kahala	–	30	–	–
GC-30094-1-32	–	30	–	–
McNair 800	–	–	30	–
L 114	–	–	30	–
G.BM 11	6	24	–	–
66d-20	15	15	–	–
G No.2043	13	17	–	–
Palmetto	–	–	16	31
GC-30279-15-8	–	–	9	21

the other hand, seeds produced by crossing soybean plants differing in IDH-phenotype (e.g. AB×BC or AB×AC) have an IDH-phenotype with 5 bands (IDH-ABC, figure). Furthermore, preliminary data for a parental cross of AB×AC yield approximately ¼ AB:½ BC:¼ AC in the F_2 progeny. These findings and the occurrence of a single IDH-band in seeds with IDH-B phenotype and 3 bands in seeds with IDH-AB, BC or AC phenotypes are consistent with the hypothesis of an *Idh* gene duplication.



Isocitrate dehydrogenase electrophoretic patterns in the cultivated soybean *Glycine max*. Horizontal electrophoresis using 12% hydrolyzed starch in citrate-phosphate buffer pH 6.8 was carried out at 80 V and 4 °C for 17 h. The agar-overlay technique was used for staining the enzyme.

The present findings in 12 varieties of cultivated soybean cannot be explained in terms of multiple alleles of a single *Idh*-locus as, for example, in the case of *Pinus rigida*[7]. On the other hand, the results concur with those found for the spadefoot toads (*Scaphiopus* spp.) which exhibit gene duplication at the fastermoving *Idh*-locus[8].

Gene duplications have been reported in a number of organisms and are represented in plants by alcohol dehydrogenase and phosphoglucoisomerase in diploid species of *Clarkia*[9,10]. The present finding of isocitrate dehydrogenase gene duplication in the cultivated soybean could facilitate the study of the evolution of the genus *Glycine*. This gene-enzyme system also serves as a useful marker for assessing the extent of natural cross-pollination, for cultivar identification and for evaluating the homogeneity of soybean cultivars.

1 A.L. Larson, Crop Sci. *7*, 311 (1967).
2 A.L. Larson and W.C. Benson, Crop Sci. *10*, 493 (1970).
3 B.R. Buttery and R.I. Buzzell, Crop Sci. *8*, 722 (1968).
4 B.R. Buttery and R.I. Buzzell, Can. J. Bot. *49*, 1101 (1971).
5 R.C. Payne and T.J. Koszyknowski, Crop Sci. *18*, 557 (1978).
6 M.B. Gorman and Y.T. Kiang, Crop Sci. *17*, 963 (1977).
7 R.P. Guries and F.T. Ledig, Heredity *40*, 27 (1978).
8 P.W. Sattler and J.S. Mecham, J. Hered. *70*, 352 (1979).
9 L.D. Gottlieb, Proc. natl Acad. Sci. USA *71*, 1816 (1974).
10 L.D. Gottlieb, Genetics *86*, 289 (1977).

Measuring cellulose decomposition using Benchkote-paper, for the estimation of soil pollution with copper[1]

K. Tatsuyama, H. Yamamoto, T. Shiota and H. Egawa

Faculty of Agriculture, Shimane University, Matsue 690 (Japan), 19 May 1980

Summary. Cellulose decomposition in paddy soil polluted by copper was determined by the use of polyethylene-backed absorbent paper (Benchkote) as a source of cellulose. It was shown that the paper is a suitable material for measuring the rate of cellulose decomposition. Correlations were found between copper content in the soil, the growth of rice plants and the cellulose decomposition rate.

A variety of soil pollutants influence microbial activities in farm land. These activities are usually assayed by chemical methods under laboratory conditions. However, the methods require much labor, and, moreover, the experimental results do not always correspond to the activity under field conditions. It is nescessary to search for some convenient and useful methods for the estimation of microbial activity in the field. In order to assay cellulase activity in soil, calico strips, cotton wool and other materials have been used as a source of cellulose[2,3]. These are in fact useful for the experiments. However, there is the possibility of loss when they are removed from the soil. In this experiment, polyethylene-backed absorbent paper (Benchkote) made by Whatman Ltd was used as a source of cellulose, and the decomposition of the cellulose in paddy soil was determined, to estimate the inhibitory effect of soil pollutants on cellulase activity.

Materials and methods. The experiments were carried out in a paddy field, under rice plant cultivation, located within easy reach of Matsue city. The field had been irrigated with

Table 1. Growth of rice plants and cellulose decomposition in paddy soil polluted by copper mine drainge

Point	Copper content in soil	Length of rice plants	Number of tillers	Remaining amount of cellulose	
				16 days	23 days
A	144.0*	48.4**	18.5**	54.9***	12.3
B	154.0	61.3	28.5	60.4	11.8
C	154.0	47.6	27.3	76.7	19.0
D	177.5	57.6	22.3	53.9	7.9
E	204.5	16.0	4.8	64.6	33.7
F	255.0	11.5	4.8	86.5	42.0
G	272.0	22.9	6.3	72.5	44.9

* µg/g dry soil. Copper was extracted with 0.1 N HCl; ** length (cm) and number: The average of 4 hills around the point; *** percent.