The regenerated plantlets showed among the normal diploid chromosome numbers (figure 3) an occurrence of polyploid ones (figures 4–6). The frequency of polyploid dividing cells was estimated to be 3.5%. In addition 6.7% of giant non-dividing nuclei which are potentially polyploids was also reported. Very high polyploid chromosome numbers, however, have not been observed in the regenerated plants in the present study due perhaps to a negative selection pressure on them in culture conditions. The present study indicates that such genetically unstable plantlets of *S. nigrum* obtained through tissue culture have potential for exploitation for crop improvement. This aspect is under further investigation.

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1. Larkin, P. J. and Scowcroft, W. R., *Theor. Appl. Genet.*, 1981, **60**, 197.
2. Shepard, J. F., Bidney, D. and Shahin, E., *Science*, 1980, **208**, 17.
3. Evans, D. A. and Sharp. W. R., *Science*, 1983, **221**, 949.
4. Larkin, P. J. and Scowcroft, W. R., *Plant cell tissue and organ culture*, 1983, **2**, 111.
5. Sun, Z, Zhao, C., Zheng, K., Qi, X. and Fu, Y., *Theor. Appl. Genet.*, 1983, **67**, 67.
6. Maddock, S. E., Risiott, R., Parmar, S., Jones, M. G. K. and Shewry, P. R., *J. Exp. Bot.*, 1985, **36**, 1976.
7. Karp, A. and Bright, S. W. J., In: *Oxford surveys of plant molecular and cell biology*, (ed.) B. J. Miflin, Oxford University Press, 1985, Vol. 2, p. 199.
8. D'Amato, F., In: *Applied and fundamental aspects of plant cell, tissue and organ culture*, (eds) J. Reinert and Y. P. S. Bajaj, Springer-Verlag, Berlin, 1977, p. 343.
9. D'Amato, F., In: *Frontiers of plant cell culture* (ed.) T. A. Thorpe, IAPTC/University Calgary, Calgary, 1978, p. 287.
10. Evans, D. A. and Sharp, W. R., *Biotechnology*, 1986, **4**, 528.
11. Murashige, T. and Skoog, F., *Physiol. Plant*, 1962, **15**, 473.

## EFFECT OF AGE ON MICRONUCLEUS FREQUENCY INDUCED BY MITOMYCIN-C IN ddY MALE MICE

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MICRONUCLEUS assay developed by Schimid and coworkers[1–4] and is being extensively used[5] as a short-term test for evaluating the mutagenicity and carcinogenicity[6–9]. The present study was planned to determine the variation in micronucleus frequency depending on the age due to mitomycin-C treatment and also to determine the suitable age of mice for sampling.

Male ddY mice (Shizuoka Agricultural Co-operative Association for Laboratory Animals, Shizuoka, Japan) of different age groups (1, 3, 6, 9 and 13-week-old) were injected with mitomycin-C (2 mg/kg b.w.) intraperitoneally. Animals of control group were injected with an equivalent volume of normal saline. The animals were sacrificed by cervical dislocation 24 hr after injection. The femoral cells were flushed out with fetal bovine serum (FBS) and smeared on clean slides. The smeared cells were fixed with methanol for 5 min and stained with Giemsa (2.5%) for 10 min. Slides were scored blindly. Micronucleated polychromatic erythrocytes (MNPCE) were examined in 1000 polychromatic erythrocytes in each animal.

The highest value of MNPCE was observed in 1-week (9.66%) and 3-week-(7.40%) old mice (table 1). In 6, 9 and 13-week-old animals the values

**Table 1** *Effect of age on micronucleus frequency due to mitomycin-C treatment*

| Age group (in weeks) | Control/ treatment | Number of animals | Polychromatic erythrocytes with micronucleus (MNPCE) Percentage ± S.D. |
|---|---|---|---|
| 1 | Control | 3 | 0.23 ± 0.17 |
| | 2 mg/kg b.w. | 4 | 9.66 ± 1.68 |
| 3 | Control | 3 | 0.07 ± 0.07 |
| | 2 mg/kg b.w. | 5 | 7.40 ± 1.55 |
| 6 | Control | 3 | 0.25 ± 1.52 |
| | 2 mg/kg b.w. | 5 | 3.79 ± 0.94 |
| 9 | Control | 3 | 0.16 ± 0.15 |
| | 2 mg/kg b.w. | 10 | 3.19 ± 0.9 |
| 13 | Control | 3 | 0.11 ± 0.15 |
| | 2 mg/kg b.w. | 4 | 3.29 ± 0.55 |

were 3.79, 3.19 and 3.29% respectively indicating that the 1 and 3-week-old animals were more susceptible than older ones. High susceptibility may be due to less developed immune system and repair capacity. The same may not be true in all strains of mice because susceptibility to environmental mutagens seems to be genetically controlled[10]. Therefore, the present study suggests that animals of each strain of atleast two age groups (preferably 3 and 9-week-old male mice) could be included for micronucleus assay. The criterion of evaluation of genotoxicity, on one age group of animals (as 8-week-old mice are generally used) does not seem satisfactory for determining clastogenicity of a substance (particularly weak clastogens).

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1. Boller, K. and Schimid, W., *Humangenetik*, 1970, **11**, 35.
2. Matter, B. and Schimid, W., *Mut. Res.*, 1971, **12**, 417.
3. Schimid, W., *Agents Actions*, 1973, **3**, 77.
4. VonLedebur, M. and Schimid, W., *Mut. Res.*, 1973, **19**, 109.
5. Heddle, J. A., *Mut. Res.*, 1973, **18**, 187.
6. Wild, D., *Mut. Res.*, 1978, **56**, 319.
7. Bruce, W. R. and Heddle, J. A., *Can. J. Genet. Cytol.*, 1979, **21**, 319.
8. Tsuchimoto, T. and Matter, B. E., *Arch Toxicol.*, 1979, **42**, 239.
9. Jenssen, D. and Ramel, C., *Mut. Res.*, 1980, **75**, 191.
10. Hayashi, M., Sofuni, T. and Ishidate, M. Jr., *Mut. Res.*, 1982, **105**, 253.

## SOME OBSERVATIONS ON PRESERVATION OF FUNGAL CULTURES BY SERIAL SUBCULTURING AND LIQUID PARAFFIN

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THE Biology Division of this Establishment maintains a collection of cultures responsible for biodeterioration of materials for research, quality control and biodeterioration testing[1]. These cultures are normally maintained by continuous growth involving subculturing which is the simplest method of maintenance for living fungi. This method is labour-intensive and time-consuming when a large number of cultures of different requirements are involved. The main disadvantages of this method are: (i) danger of variation in physiological and morphological characteristics, (ii) problem of contamination by air-borne spores or mite carried infections[2], (iii) requires constant specialist supervision to ensure purity of the original culture. The interval between transfers depends mainly on various physiological requirements of the particular fungus and to some extent on external conditions especially on storage temperature. It may vary from a few weeks to 6–12 months. Storage at a lower temperature at about 5°C can extend the transfer interval of most of the fungi[3,4]. Methods of preservation of a particular culture/strain collection depends on several factors viz type of cultures, purpose of preservation, duration of storage, source of isolation, available resources and so on. It is well known that the storage life of cultures can be increased by several methods by reducing its metabolic activity to a minimum. Therefore, an attempt was made to find out the effect of extended transfer period on drying and the viability of a few fungal cultures maintained at $6\pm2$°C with and without liquid paraffin.

Twenty-one cultures viz *Actinomyces* sp. (Culture No. 1), *Aspergillus amstelodami* (2), *A. fumigatus* (3), *Chaetomium globosum* (6), *Penicillium citrinum* (8), *Polystictus hirustus* (11), *Macrophomina phaseoli* (25), *Diplodia* sp. (28), *Memnoniella echinata* (30), *Oospora* sp. (32), *Verticillium glaucum* (34), *Byssochlamys* sp. (35), *Botryodiplodia theobromae* (36), *Cephalothecium* sp. (38), *Hapluria* sp. (40), *Aspergillus oryzae* (42), *Paecilomyces* sp. (43), *Curvularia* sp. (64), *Penicillium* sp. (69), *Trichoderma* sp. (70) and *Aspergillus sydowi* (76)