SELECTION AND CHARACTERIZATION OF METHIONINE-SULFOXIMINE RESISTANT CELL CULTURES OF BRINJAL (*SOLANUM MELONGENA* L.)

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RECENTLY, there have been several reports where cultured plant cells have been used to isolate cell lines resistant to various stress conditions, *viz* amino-acid analogues, herbicides, salinity etc[1,2]. Such resistant cell lines, besides being useful for plant improvement, can also be used as biochemical markers in somatic cell fusion experiments[3,4]. This communication reports the selection and partial characterization of brinjal cell cultures resistant to methionine-sulfoximine, an analogue of glutamine. The calli were initiated from leaf petiolar tissue of brinjal (*Solanum melongena* Linn cv Pusa Purple Round) on Gresshoff and Doy's[5] basal medium supplemented with 1 mg/l each of 6-benzyladenine, 2, 4-dichlorophenoxyacetic acid and 1-napthaleneacetic acid (DGJ medium). 1 g callus pieces were agitated in the liquid medium of same composition to initiate cell suspension cultures. To maintain the suspension cultures, 10 ml aliquots were inoculated into 100 ml of fresh medium at an interval of 20 days. To induce mutations, ethylmethane sulphonate (EMS) treatment was carried out as described by Widholm[6]. Resistant cell lines were isolated by plating EMS treated and untreated cell suspension cultures on DGJ medium in petri plates containing 1 μM of DL-methionine-DL-sulfoximine (MSO). The petri plates were sealed with parafilm-M (American Can Company) and incubated at 27 $\pm$ 1°C for 2 months. To test the stability of resistance, the isolated clones were grown for 2 months on normal medium before bringing them back to stress medium. Since the suspension cultures comprised cell aggregates rather than the single cells, the number of cells in a known fresh weight was determined using chromic acid cell separation method[7]. Assuming that a resistant clone is derived from a single cell, the frequency of resistant cells (f) was calculated as $f = n/N$, where n is the number of resistant clones and N the total number of cells. Growth studies were conducted by inoculating 0.5 g of cells from actively growing cell suspension in DGJ liquid medium containing different concentrations of the analogue and subsequently determining an increase in fresh weight after 20 days of incubation on a low speed shaker. Using the method of Barnett and Naylor[8] free amino-acids were extracted from exponentially growing cells, which had been cultured in the absence of the analogue for 4 months. Colorimetric methods were used to estimate total free amino-acids[9] and free methionine[10]. Free glutamine was estimated by two-dimensional paper chromatography[11].

MSO-resistant clones were isolated from EMS treated as well as untreated cultures. However, the frequency of resistant clones was considerably more in EMS mutagenised cultures (1.3×10^{-7}) as compared to the control (4×10^{-8}). The resistance of the isolated clones was stable in the absence of the analogue. The inhibitory effects of MSO on one of the resistant cell line and the wild type cells is shown in figure 1. In a 20-day experiment, the growth of the normal brinjal cells was completely inhibited at 0.5 μM MSO, whereas the MSO-resistant cell line required as much as 7.5 μM of the analogue for growth inhibition. Since most of the amino-acid analogue resistant plant cell lines have been reported to over-synthesize the corresponding natural amino-acid[12], the free amino acid levels were determined in these lines (table 1). The MSO-resistant cells contained 599 nmoles free glutamine/100 mg dry weight, while the normal line had 163 nmoles. Such an *accumulation of the corresponding amino-acid may*

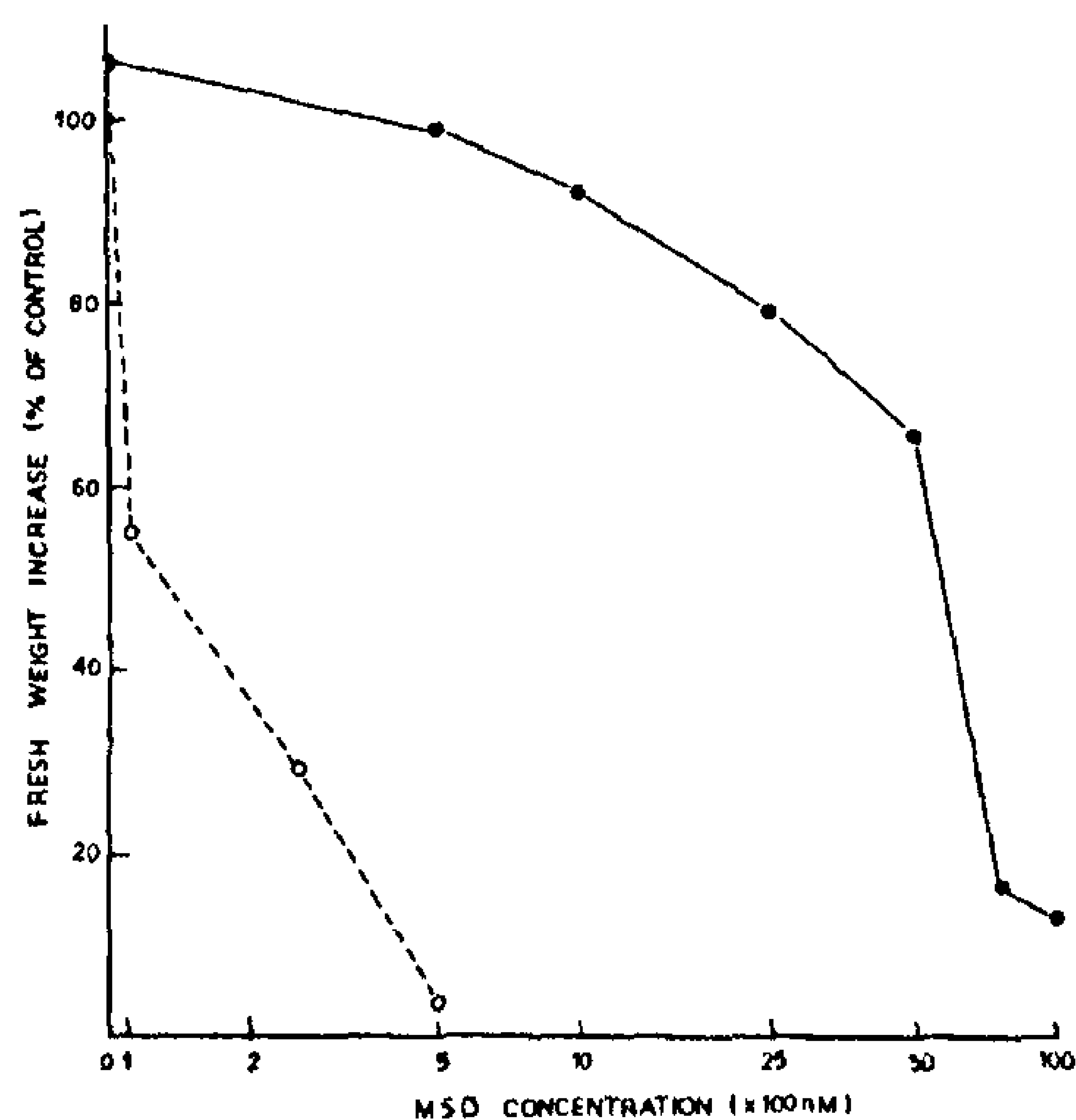

Figure 1. Effect of MSO on growth of MSO-resistant (—●—) and wild type (---O---) cells of *Solanum melongena* after 20 days of incubation in liquid media. The results are the average of three replicates.

Table 1 *Comparison of total free amino acids, free methionine and free glutamine contents in MSO-resistant and wild type cells of Solanum melongena*

	MSO-resistant	Wild type
Total free amino-acids (μmol 100 mg dry weight)	22.3 ± 0.7	12.0 ± 1.7
Free methionine (nmol 100 mg dry weight)	36.3 ± 2.8	30.0 ± 2.0
Free glutamine (nmol 100 mg dry weight)	599.0 ± 25.6	163.0 ± 10.8

be the result of a relaxed feedback inhibition of the control enzyme, which seems to be the major reason of resistance to analogues[12]. The resistant cells also showed a higher pool size of total free amino-acids as compared to control. Carlson[13] reported accumulation of free methionine in MSO resistant cells of tobacco. In the present study, however, there was no accumulation of free methionine in MSO resistant brinjal cells as MSO is an analogue of glutamine and not methionine[14]. The possible use of these resistant cell lines in somatic cell hybridization with other *Solanum* species is the subject of further experimentation.

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REGENERATION OF PLANTS FROM CALLUS CULTURES OF *ANTHURIUM PATULUM*

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PROPAGATION of ornamental plants using *in vitro* techniques has received considerable attention in recent years[1]. *Anthurium patulum* is an exotic grown for its attractive foliage. The conventional method of propagation of this plant is rather slow. The plants have hastate, shiny, dark green leaves with spreading basal lobes with pale green veins and greenish brown spathes. The present note reports the regeneration of *A. patulum* plants from callus cultures.

Young leaves, petiole, pedicel and spathe excised from 3-year old flowering plants were surface-sterilised in 70% ethanol for 30 sec, followed by 5 min in 0.1% $HgCl_2$ with 0.25 ml/l of tween 20. The explants were washed thrice with sterile deionized water. Leaf and spathe were cut into 1.5 cm^2 pieces whereas petiole and pedicel were cut into 2 cm pieces and aseptically cultured on a basal medium (BM) comprising mineral salts of Murashige and Skoog[2] at half strength except Fe-EDTA, vitamins of Lin and Staba[3], 0.6% agar (SISCO, bacteriological grade) and 3% glucose at pH 5.8. Growth substances such as 2,4-D (2,4-dichlorophenoxyacetic acid), BA (6-benzylamino purine), Kn (kinetin), Z (zeatin) and 2ip 6-(3-methyl-2 butenylamino) purine were added to BM at various concentrations. The cultures were incubated in the dark at 25 ± 2°C at 55–60% RH. Each experiment was conducted with 12 replicates and was repeated six times.

Leaf, pedicel, spathe and petiole segments produced pink coloured callus on BM + 2,4-D (0.1 mg/l) + BA (1 mg/l) within 6 weeks of incubation (figure 1A). The intensity of callus and the frequency was highest in leaf segments cultured with mid-veins (table 1). After 6 months of culture which involved 3 passages of subculture on fresh medium every two months, differentiation of pink shoots was observed (figure 1B).