

**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 ( $\mu\text{mol}$ 100 mg dry weight)	22.3 $\pm$ 0.7	12.0 $\pm$ 1.7
Free methionine (nmol 100 mg dry weight)	36.3 $\pm$ 2.8	30.0 $\pm$ 2.0
Free glutamine (nmol 100 mg dry weight)	599.0 $\pm$ 25.6	163.0 $\pm$ 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<sup>12</sup>. The resistant cells also showed a higher pool size of total free amino-acids as compared to control. Carlson<sup>13</sup> 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<sup>14</sup>. 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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1. Maliga, P., *Int. Rev. Cytol.*, 1980, 11A (Suppl.), 255.
2. Widholm, J. M., *Plant cell culture in crop improvement*, (eds) S. K. Sen and K. L. Giles, Plenum Press, New York, 1983, p. 71.
3. White, D. W. R. and Vasil, I. K., *Theor. Appl. Genet.*, 1979, 55, 107.
4. Harms, C. T., Potrykus, I. and Widholm, J. M., *Z. Pflanzenphysiol.*, 1981, 101, 377.
5. Gresshoff, P. M. and Doy, C. H., *Planta*, 1972, 107, 161.
6. Widholm, J. M., *Can. J. Bot.*, 1976, 54, 1523.
7. Steward, F. C. and Shantz, E. M., In: *The chemistry a mode of action of plant growth substances*, Science. London, 1955, p. 165.
8. Barnett, N. M. and Naylor, A. W., *Plant Physiol.*, 1966, 41, 1222.
9. Yemm, E. W. and Cocking, E. L., *Analyst*, 1955, 80, 209.
10. Horn, M. J., Jones, D. B. and Blum, A. E., *J. Biol. Chem.*, 1946, 166, 313.

11. Stepka, W., *Methods Enzymol.*, 1957, 3, 504.
12. Widholm, J. M., *Crop Sci.*, 1977, 17, 597.
13. Carlson, P. S., *Science*, 1973, 180, 1366.
14. Meins, F. Jr. and Abrams, M. L., *Biochim. Biophys. Acta.*, 1972, 266, 307.

## REGENERATION OF PLANTS FROM CALLUS CULTURES OF *ANTHURIUM PATULUM*

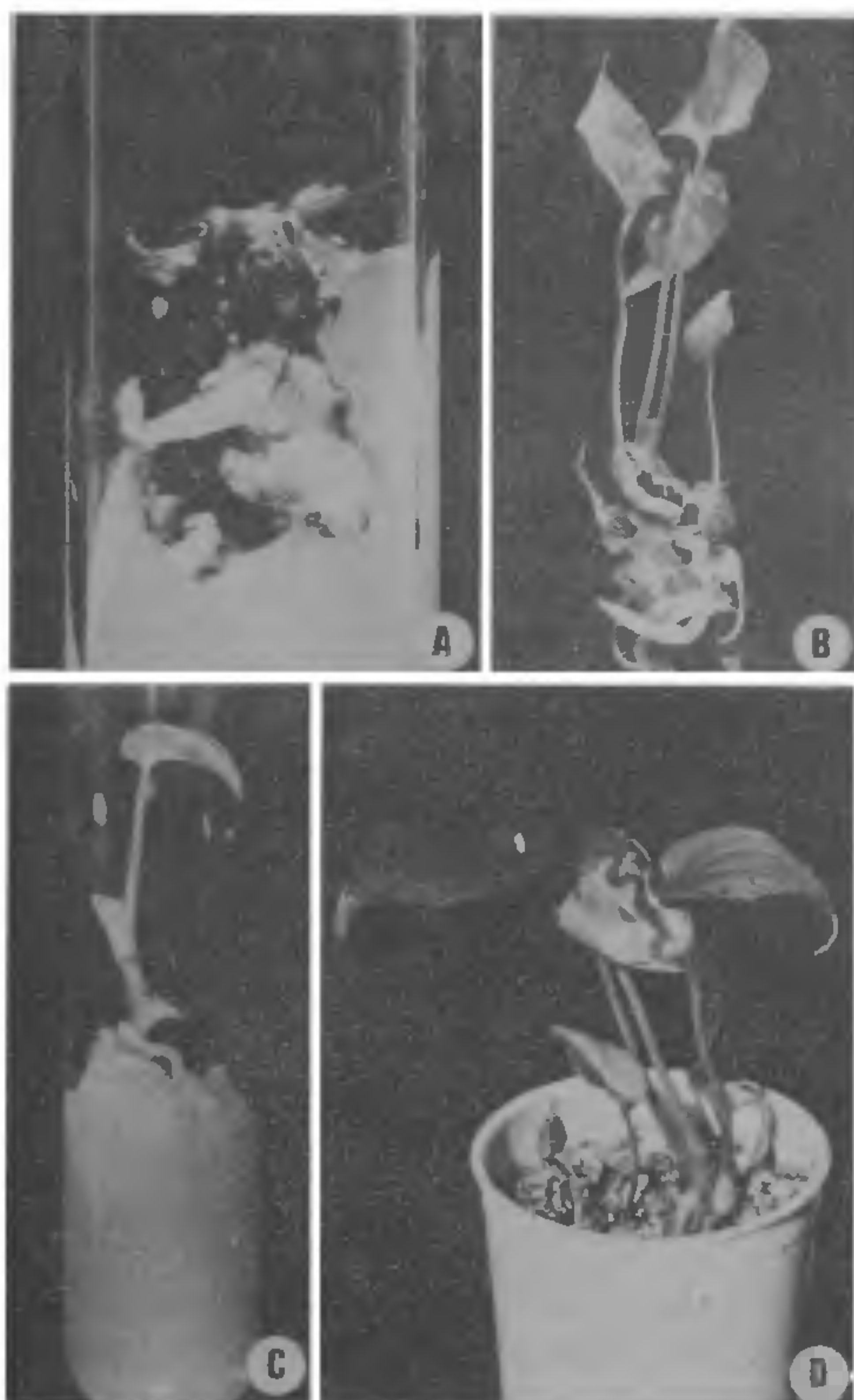
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PROPAGATION of ornamental plants using *in vitro* techniques has received considerable attention in recent years<sup>1</sup>. *Anthurium patulum* is an exotic grown for its attractive foliage. The conventional method of propagation of this plant is 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%  $\text{HgCl}_2$  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<sup>2</sup> 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<sup>2</sup> at half strength except Fe-EDTA, vitamins of Lin and Staba<sup>3</sup>, 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  $\pm$  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).



**Figures 1A–D.** Stages in plant differentiation from callus cultures of *Anthurium patulum*. **A.** Leaf explant showing callusing on BM+BA (1 mg/l)+2,4-D (0.1 mg/l) at the end of 6 weeks incubation. **B.** Regeneration of shoots and roots from callus on BM+BA (1 mg/l)+2,4-D (0.1 mg/l). **(C)** An isolated shoot on BM+NAA (1 mg/l) showing root development. **D.** Regenerated plants of *A. patulum* growing in plastic cups with vermiculite.

Calli originating from petiole lacked the ability to produce shoot buds unlike the situation in *A. andraeanum*<sup>4</sup>. To optimise conditions for shoot bud and plant regeneration, experiments were conducted with other cytokinins (2 iP, Z, Kn and BA at 0.1, 1 and 2 mg/l) in combination with 2,4-D (0.1 mg/l). Leaf segments cultured on these media included mid-veins. Kn and 2ip (1 mg/l) stimulated shoot differentiation to a lesser degree while zeatin was ineffective. In *A.*

**Table 1** Responses of explants of *Anthurium patulum* grown on BM + BA (1 mg/l) + 2,4-D (0.1 mg/l).

Explant source	Extent of callus development	Frequency of callus development (%)	Mean number of shoot buds/culture $\pm$ SD
Leaf with mid-vein	+++	83.3	7.4 $\pm$ 1.7
Leaf devoid of mid-vein	++	80.4	7.1 $\pm$ 1.1
Pediceal	++	41.6	5.7 $\pm$ 0.9
Spathe	++	55.9	5.4 $\pm$ 1.0
Petiole	++	34.2	0

Intensity and frequency of callus development were determined at the end of first serial passage and the number of shoot buds at the end of third serial passage. Average of 12 replicates. ++, good; +++, excellent. SD: standard deviation

**Table 2.** Effect of different cytokinins on callus development and shoot bud regeneration.

Cytokinin (1 mg/l)	Extent of callus development	Frequency of callus development (%)	Mean number of shoot buds/culture $\pm$ SD
2, iP	++	50.0	2.9 $\pm$ 0.5
Kn	+	41.6	3.4 $\pm$ 0.6
BA	+++	83.3	7.4 $\pm$ 1.2
Z	0	0	0

Average of 12 replicates; +, moderate; ++, good; +++, excellent; SD: standard deviation.

*andraeanum*<sup>4</sup> Z promoted shoot bud formation. In the present study the best response was obtained with BA (1 mg/l) + 2,4-D (0.1 mg/l) (table 2). The capability of calli to produce shoots was maintained even at the end of 2 years of subculture. All cytokinins (at 2 mg/l) proved inhibitory.

The shoot buds produced roots on the same medium but root growth was more prominent in shoots transferred to BM+naphthaleneacetic acid (NAA, 1 mg/l) (figure 1C). The regenerated plants were transferred to plastic cups with vermiculite (figure 1D) and were irrigated with a nutrient solution containing MS salts at half strength. Plants were covered with a bell jar to maintain a humid condition and were planted in the nursery after 3 weeks. Forty plants were transferred to the nursery of which 75% survived, flowered and were similar to the parental material.

The procedure developed by us should be beneficial



for micropropagating and introducing native anthuriums abundant in the forests of Kerala State.

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1. Holdgate, D. P., Reinert, J. and Bajaj, Y. P. S., In: *Applied and fundamental aspects of tissue and organ culture*. Springer Verlag, Berlin, 1977, p. 18.
2. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, 15, 473.
3. Lin, M. and Staba, E. J., 1961, *Loydia*, 24, 139.
4. Pierik, R. L. M., Van Leewan, P. and Ritger, G. G. C. M., *Neth. J. Agric. Sci.*, 1979, 27, 221.

## STUDIES IN NEMATOPHAGOUS FUNGI: IX *MYZOCYTIUM PAPILLATUM*—A NEW RECORD FROM INDIA

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IN studies of the nematophagous fungi of Varanasi, a species of *Myzocytiium* was encountered which has not been previously reported from India. *Myzocytiium*, a member of Oomycetes is found parasitic on nematodes. Barron<sup>1</sup> described 6 species of the genus *Myzocytiium* from Canada. An interesting feature of the genus is that almost all the species exhibit different modes of parasitic cycle, which range from primitive to advanced Oomycetous type.

The present note deals with the detailed account of the chief structural characteristics of *Myzocytiium papillatum* Barron. Nematodes were isolated from soil using Cobb's sieving and decanting technique<sup>2</sup>. Using Giurma and Cooke's method<sup>3</sup>, nematode suspension was concentrated at 1000 RPM for 3 min. The supernatant was discarded and resuspended in a few ml of distilled water. On immediate examination of the suspension, few nematodes showed symptoms of fungal attack. After four days or more of incubation in

distilled water, they were again centrifuged. After discarding the supernatant, the nematodes were poured on fresh water agar plates<sup>4</sup>. They were again incubated at  $25 \pm 2^\circ\text{C}$  and then a large number of nematodes were found to be infected.

*Myzocytiium papillatum* Barron.

Zoospores lens-shaped, laterally biflagellate,  $4.2 - 6.3 \mu\text{m}$  long. The zoospores encyst on the nematode cuticle before penetration and infection. The infection thallus formed from encysted spore, grew on the entire length of the host. Sporangia measured  $21.0 - 37.8 \times 16.8 - 20.6 \mu\text{m}$  often lemon shaped, distinctly papillate at one or both ends, linearly arranged in the nematode body. Evacuation tubes  $8.4 - 12.6 \times 6.3 - 8.4 \mu\text{m}$  produced singly from zoosporangia.

Two adjacent segments behave as antheridia and oogonia. They are similar in appearance to that of sporangia. Oospores are thick-walled, smooth, and spherical with  $16.8 - 21 \mu\text{m}$  in diameter.

The fungus was isolated from the soil under Papaya vegetation during March and April 1984.

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1. Barron, G. L., *Can. J. Microbiol.*, 1976, 22, 752.
2. Cobb, N. A., *Agric. Tech. Circ. Bur. Plant Ind. U. S. Dep. Agric.*, 1981, 1, 48.
3. Giurma, A. Y. and Cooke, R. C., *Trans. Br. Mycol. Soc.*, 1972, 59, 213.
4. Barron, G. L., *Can. Biol. Publ. Ltd.*, Ontario, Canada, 1977.