

Fig. 2. Sample recordings from system.

A) Upper trace muscle action potential resulting from nerve stimulation, lower trace mechanogram. Time cal. 10 msec.

B) As above, time cal. 2 msec. Sensitivity of mechanogram increased to facilitate determination of start of contraction (arrow) in relation to MAP. Ripple in mechanogram is carrier frequency of transducer.

minus 6 volts are required. We used a stabilized power source, but a battery would also do. To protect the transducer from excessive voltage, and especially from being connected with the wrong polarity, we wired a zener diode into the system as shown. We also attached a grounding collar to the transducer to keep the carrier frequency out of the output. The output signal is connected directly to the DC input of the second beam of the CRO.

Discussion. The construction of this recording apparatus proved to be relatively uncomplicated. Care must be taken, however, in selecting and installing the guide surfaces and in using relatively supple transmission string; these are the principal points where recording inaccuracies can be introduced. The frictional errors from the transducer itself are minimal as long as the device is level. In terms of performance, it fulfilled our requirements. The nerve-muscle preparations usually maintained their initial physiological state for 2 or more hours in the moist chamber, especially if they were not unnecessarily stimulated. The mechanical recordings were of high sensitivity, stable and could faithfully follow up to the tetanus frequency of the preparation. To test the accuracy of the measurement of the latency of the myogram, we intentionally loosened in several experiments a recording electrode. The resulting movement artefact in the electrical recording always agreed in time with the onset of the myogram. Although we used a dual beam oscilloscope for simultaneous viewing of the two output signals, a single beam CRO would also suffice for alternate examination. This device has stood the test of repeated use. The principal cost, aside from construction time, is that of the transducer. This however is not altered during the construction and can be utilized for other purposes at any time or be placed in a new frame should the old one be damaged.

## Vegetative Propagation of the Cactus Mamillaria woodsii Craig Through Tissue Cultures

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Summary. A new method of clonal multiplication is described in a representative from the Cactaceae family, which offers wide experimental and horticultural applications.

The methods of tissue cultures provide large possibilities for rapid and mass multiplication of plants. Among the techniques successfully applied in vegetative propagation in vitro belong, e.g., the cultures of stem tip meristems, axillar buds, bulb scales, and callus cultures with induced organogenesis. Using the techniques of clonal multiplication of ornamental plants through shoot regenerants from callus cultures, considerable results were obtained in the genuses Freesia<sup>2</sup>, Gazania<sup>3</sup>, Crassula and Kalanchoe<sup>4</sup>, Chrysanthemum, Dianthus, Euphorbia, Pelargonium, Asparagus, Haworthia, Saintpaulia, Gladiolus, Passiflora, etc. (see for review Murashige<sup>5</sup>). As far as we are informed, no report was published concerning the above-mentioned propagation techniques in plants of the Cactaceae family.

Material and methods. Starting material for our experiments were plants of Mamillaria woodsii species, Cactaceae family. 2-year-old plants of 1.5-2.0 cm in diameter were lifted from the soil including roots and thoroughly washed in running water. Afterwards the roots were removed and the stems were sterilized applying different

techniques. The most promizing sterilization method proved to be the application of 70% ethanol in which stems were submerged for 5 min. For another 5 min the stems were put into 3% chloramin B solution with a drop of detergent and finally they were given triple rinse in sterile distilled water. From stems prepared by this manner, transverse segments of the width of approx. 3 mm were cut in a sterile box. From the cuttings the central vascular bundle was removed and the cuttings were divided into 4–5 segments to avoid in the explants the occurrence of the secondary vascular bundles reaching up

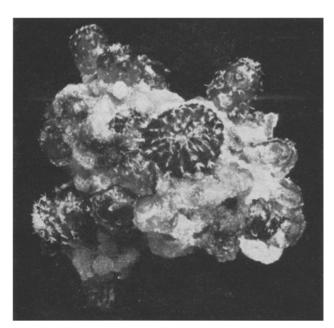
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<sup>&</sup>lt;sup>2</sup> Y. P. S. Bajaj and R. L. M. Pierik, Neth. J. agric. Sci. 22, 153 (1974).

<sup>&</sup>lt;sup>3</sup> B. Landová and Z. Landa, Experientia 30, 832 (1974).

<sup>&</sup>lt;sup>4</sup> R. L. Jones and T. Murashige, Am. J. Bot. 60, Suppl. 4 (1973).

<sup>&</sup>lt;sup>5</sup> T. Murashige, A. Rev. Plant Physiol. 25, 135 (1974).



The formation of shoot regenerants in callus cultures of cactus Mamillaria woodsii Craig.

to the individual areoles. Finally, the epidermis together with the rests of prickles were cut off from each segment so that only the pith tissue was left from the stem.

The explants were then placed into Erlenmeyer flasks containing synthetic agar medium prepared according to Murashige and Skoog 6 with 2.0 mg  $\cdot$  1<sup>-1</sup> of  $\beta$ -indolylacetic acid, and 2.0 mg  $\cdot$  1<sup>-1</sup> of kinetin. The flasks were stored in a cultivation chamber and lit continuously by white fluorescent tube light at the temperature of 25  $\pm$  3 °C.

Results and discussion. After approximately 1 week, the colour of explants was changed. Some of them went brown and were affected by necrosis, while in others the colouring changed to red of different shades to violet; after a few days of stagnation the latter started to produce callus, which in turn lost its reddish coloration and turned a light green. After 5 weeks of cultivation, the calluses were strong enough to be subcultured in a fresh medium, the composition of which was the same as shown above. After subcultivation an organized growth producing flat cactus shoots with apparent areoles was

observed in some callus tissues. Later on the plants started shooting from areoles and subsequently whole tufts of cactus shoots with tiny prickles were produced (Figure). In one case even formation of rootlets was observed. In other cultures the callus continued growing in an unorganized way. After a 10 week's period, the other subculture could be divided into 3 groups. In one, differentiated plants from callus were produced, in the 2nd only well growing calluses without any traces of organogenesis were observed. The calluses produced in the 3rd group turned brown and were affected by necrosis.

Well differentiated cactus shoots 1 cm long were extirpated from the cultures. The cut was treated with a commercial rooting stimulator, and when dry the regenerants were placed in bowls containing humid perlite. Approximately after 4 weeks of cultivation, roots started to grow from the cut.

Mamillaria woodsii Craig thus extends the list of species in which formation of regenerants by controlled organogenesis in callus cultures in vitro was achieved. The synthetic medium chosen for the above-mentioned experiment is being used in our laboratory in routine work for induced organogenesis in the calluses of Nicotiana tabacum. When comparing these 2 plant species, it can be concluded that the identical nutrition and hormone conditions were well acceptable even for such systematically and ecologically remote plants as the cacti are. In order to increase the production of regenerants, the explants should be subcultivated first on media stimulating rapid and rich callus growth (e.g., by addition of 2,4-dichlorophenoxyacetic acid) with subsequent subcultivation on the media inducing organogenesis. It would perhaps be possible to obtain still better growth conditions by modifying the basal Murashige and Skoog<sup>6</sup> medium. By a proper balance of the synthetic medium some unfavourable effects, e.g., necrosis and anthocyanescence, could probably be avoided.

Mamillaria woodsii may be used as an example demonstrating rapid and mass propagation of selected genotypes in cacti. The development period of regenerants in tissue cultures is at least 1 year shorter as compared with the vegetation period of plants obtained from seeds. Moreover, the production of virus-free plants from tissue cultures of cacti may be of great significance. If the above clonal multiplication method is improved and adopted to other species of the Cactaceae family, it might be recommended for horticultural practice as commonly used tissue cultures in vitro in orchids or carnations.

<sup>6</sup> T. Murashige and F. Skoog, Physiologia Plant. 15, 473 (1962).

## Brain Chopping: A New Method for the Rapid Removal of Newborn Rat Brain<sup>1</sup>

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Summary. A new multi-bladed air-driven guillotine is described. This device removes newborn rat heads and slices them into several thin wafers, which facilitates freezing and permits easy regional dissection.

In neurochemical investigations it is frequently desirable to measure both labile metabolites and enzymes in the same sample from various regions of the brain. Decapitation or submersion of the intact animal in liquid nitrogen results in a delay of many seconds before subcortical brain regions freeze <sup>2–5</sup>, which may result in significant

- <sup>1</sup> Supp. in part by U.S. Public Health Service Grant . AA-01391.
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