

suffer heavy losses because of virus disease and insects are the main cause for spreading the virus. Accurate identifications of these insects and acarines are very essential for any control measures to be envisaged. No doubt, some of these problems are tackled by the Veterinary Research Institute and the National Institute for Communicable diseases, but a separate wing to deal with problems of taxonomy relating to veterinary and medical entomology within the National Institute would be highly desirable.

Concluding this review, the author would like to emphasise that taxonomy has been badly neglected in India due to the lack of encouragement and support of administrators. Without accurate identifications any study on zoological material becomes meaningless. Insect systematics must be recognised as important to the development of agriculture in general and food production in particular. Young entomologists should take more and more to the study of systematics. To quote Ghorpade<sup>15</sup>, Taxonomists have a solemn responsibility to carry out work of a high international standard and to offer their expertise whenever and wherever it is required. Only by maintaining close contact and co-operation among themselves can they help serve mankind and justify their work.

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## PLANT MERISTEMS AS MONITORS OF GENETIC TOXICITY OF ENVIRONMENTAL CHEMICALS

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### ABSTRACT

The methods involved in using root meristems for monitoring the genetic damage by chemical environment are described. These include certain prerequisites preceding testing, protocols of assay for cytotoxicity, mitotoxicity and clastogenicity of the chemicals, processing meristems and sampling required at different stages. The meristem assay may be preferentially used in certain contingencies and universally employed as a first tier short-term screening method in genetic toxicology.

### INTRODUCTION

THE utility of plant monitors in the genetic toxicology of environmental chemicals has been widely recognised<sup>1,2</sup>. Of these, meristems were more

prominent owing to their continuous use ever since Levan<sup>3</sup> developed the *Allium* test. However, much importance has not been accorded to the meristems due to the disrepute generated by an often inconsistent

and erratic use, notwithstanding the accumulation of enormous information by using them<sup>4</sup>. Therefore, there is a need to redefine the meristem procedures, as they are likely to be in continuous use due to their amenability to minimal facility-resources and for *in situ* monitoring besides other obvious advantages<sup>5</sup>. Though meristems of several plants had been in use<sup>1</sup>, due to a relative preponderance of literature, *Allium cepa* is chosen as an example to describe the protocol developed from an experience with pesticides and alkaloids in various systems. Since the earlier reviews<sup>6,7</sup> were devoted to other purposes, it is probably the first time that this mitotic system is described to draw reproducible information on the genetic toxicity of chemical substances.

#### MATERIALS AND METHODS

Onion bulbs are maintained in the field, year after year. Following a thorough cleansing, they are placed on tubes, keeping the root eyes in touch with the water within. Roots are struck within 2-3 days.

#### Prerequisites:

(a) *Assay system*: Mitotic frequencies are the highest in the 2-3 cm long roots and between 0600-0900 hr. Duration of cell cycle has been found to be 14 hr by the colchicine method. The experimental work is therefore planned in accordance with these findings.

(b) *Test compounds*: The compounds are tested for solubility and a solvent, wherein it can dissolve in the minimum quantity, is used. The stock solution and further dilutions are made in a phosphate buffer of pH 7. Test solutions are periodically replaced by fresh ones to compensate for the loss of stability, if any. Treatments are done in dark, to prevent photolysis of some compounds and recoveries are carried out in Hoagland's or Knopp's nutrient media<sup>8</sup>. Air is periodically bubbled into the tubes. A range of strengths are arbitrarily chosen to determine (a) cytotoxic concentration, (b) cytotoxic threshold, (c) minimum effective concentration and (d) an additional one between (b) and (c), for dose-response assessment.

#### Assay for Cytotoxicity:

Cytotoxicity is suggested by mitodepression and nuclear pycnosis. Minimum effective concentration, cytotoxic threshold and pycnotic strength are determined in this assay which provide guidelines for further work. Data are scored as mitotic indices of dividing cells.

(a) *Chronic exposure and recovery for one cell cycle*: The cytotoxic concentration causing nuclear pycnosis after exposure for a full cell cycle is the upper

limit of testing. The meristem regions of intact roots are immersed in the test solutions, for a full cell cycle and its fractions *i.e.* 1 hr (M), 3 hr (G<sub>2</sub> onwards), 6 hr (late S onwards), 9 hr (early S onwards) and 14 hr (G<sub>1</sub> onwards). These fractions constitute the duration of expected impact on cells beginning from the stage of cell cycle given in the parentheses. In other words, if meristems are treated for 3 hr (G<sub>2</sub>) it would mean that (a) the difference between the stage of examination (M) and G<sub>2</sub> is about 3 hr and (b) the effects scored here are essentially caused in cells passing from G<sub>2</sub> to M. Meristems exposed for 14 hr, are recovered for slightly more than a cell cycle duration, (*i.e.* 15 hr) to see if mitotic indices revert to the control frequency. In lower strengths the meristems usually regain normalcy but in the highest, they do not. In between, the recovery will be only partial. Thus the concentration, where 50% mitodepression is still retained is taken as the cytotoxic threshold. Meristems exposed only to fractions of cell cycle *i.e.* 1-9 hr indicate dose responses and the minimum effective concentration.

#### Assay for Mitotoxicity:

Mitotoxicity is suggested by the dysfunction of spindle and phragmoplast. Thus the ability of the chemicals to cause aneuploidy and polyploidy is tested in this assay. Data are presented as frequencies of abnormal cells, *i.e.*, per cent of arrested metaphases, and so on.

(a) *Spindle dysfunction* (Pulse exposure and one cell cycle recovery): The meristems are exposed for 3 hr which was found adequate to accumulate arrested metaphases, if caused, and recovered for a full cell cycle duration, to check if tetraploid metaphases occur<sup>8</sup>. If they do not occur the effect is transitory being due to respiratory asphyxiation. Anaphases are screened for laggards and nondisjunction which indicate partial spindle dysfunction and appear as aneuploid cells upon recovery.

(b) *Phragmoplast dysfunction* (Pulse exposure and one cell cycle recovery) The meristems are exposed for 4 hr., which was found adequate to accumulate binucleate cells, if caused, and recovered for a full cell cycle duration to check if bimitoses occur<sup>8</sup>. Unless the latter also are caused, the effect is considered transitory.

#### Assay for Clastogenicity (Pulse exposure and periodical recovery):

Clastogenic manifestations of chromosomes such as gaps, fragments, micronuclei, bridges and exchanges suggest toxicity to chromosomes. Information on the ability of the compound to effect the structural integrity at chromosomal, chromatid and subchromatid levels and whether these effects appear immediately in

the shorter recoveries or delayed until later is obtained in this assay. The results are presented as the frequency of damaged cells, i.e. % clastoant cells.

#### Processing Meristems:

Meristems are fixed in Carnoy's fluid for 15 min, stored in 70% alcohol, feulgen-stained and squashed in 10% acetic acid. Observations and microphotographs are taken from temporary slides.

#### Sampling:

Sampling is made as random as possible. For every variable of the experiment 10 meristems are selected from 10 bulbs and from each meristem 2-4 slides are prepared. In each slide, 10 microscopic focusses are screened for various endpoints mentioned earlier. Thus, the frequency of each endpoint per root is obtained. From such frequencies in the 10 meristems, a mean frequency is calculated. Water controls with traces of solvent of the test compound are simultaneously maintained and assayed likewise. The data are subjected to student's *t*-test.

#### DISCUSSION AND CONCLUSION

Mitotoxicity and clastogenicity of environmental chemicals ultimately lead to numerical and structural alterations in the karyotype which may therefore be termed together as karyotoxicity.

The protocol described may apply to all meristem systems necessitating only marginal adjustments. In the case of undefined industrial effluents, the bulbs may be directly grown over these complex mixtures and their dilutions. If roots are not struck, this can be taken as a measure of cytotoxicity. If the roots are initiated, the meristems can further be grown for about 48 hr in the nutrient medium to test for mitotoxic and clastogenic manifestations. They can also be grown in polluted habitats for *in situ* monitoring.

While the value of the meristem assay in genetic toxicology is not sought to be exaggerated<sup>5,10</sup>, it may be pertinent to remark that in the animal systems the mitotoxic endpoints cannot be easily monitored due

to a generally accompanying lethality. Also, certain kinds of substances like organophosphates and industrial effluents are so toxic in animals that the genetic damage cannot reliably be screened. Further these are not amenable for *in situ* screens. Therefore the plant monitors are preferred in such situations. This contention is further strengthened by the recent demonstration<sup>12</sup> of correspondence between plant and mammalian responses to environmental chemicals.

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