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HIGHER PLANTS AS CYTOGENETIC MONITORS FOR CHEMICAL AGENTS[1]

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I am indeed grateful to theIndian Botanical Society and to my fellow botanists for the award of the Birbal Sahni medal. However, I am still of the opinion that I do not deserve this signal honour to the extent several other botanists of our country do.

Professor Birbal Sahni has been one of the most outstanding personalities in Indian Science and indeed in World Botany. As a student in Intermediate classes, as early as in 1946-47, we were given the details of his memorable contributions in the reconstruction of *Williamsonia sawardiana*, by our teacher, Professor K. M. Gupta, who was one of the earliest DSc's under Professor Sahni. Perhaps this makes me an intellectual grandchild. At least I hope so, and can only feel proud that this award has been given to me.

Since it is customary to speak on one's own contributions during an award lecture, there were two options open to me : (i) to speak on cytology as related to taxonomy and environmental factors in various plant groups or (ii) on the genotoxic effects of various groups of chemicals, particularly environmental agents. I chose the latter because it has a wider perspective in the present context and is suitable for a general gathering.

MUTATIONS—TYPES OF:

The interaction of chemical agents with the genetic material of the living cell (DNA, chromosomes) may lead to both qualitative and quantitative alterations resulting in immediate and inherited modifications (mutations, chromosomal alterations) of the genotype and the phenotype. A mutagenic chemical may be identified by its property of inducing, either directly or following metabolic activation, mutations, or increasing significantly the mutation rates above the spontaneous background level. Mutations involve molecular changes in DNA structure (Table I), either direct or indirect (Table II).

MUTAGENS—TYPES OF:

In general, identified environmental mutagens have been categorised into three loose groups according to their chronological appearance (Sharma, 1984).

a) the naturally occurring substances, such as the alkaloids and flavonoids in plants and mycotoxins. The evolution of the animal kingdom has proceeded with continuous exposure and subsequent adaptation to these mutagens. For example, human beings have been exposed continuously to the flavonoids, quercetin and kaempferol and their glycosides, rutin and astragalin present in edible plants.

b) the products of pyrolysis, which were introduced into the environment with the discovery of fire about 10^5 years

1. Address delivered on the occasion of award of Birbal Sahni Medal at Jaipur during VII All India Botanical Conference in December, 1984.

TABLE I

CLASSIFICATION OF MOLECULAR CHANGES IN DNA DUE TO MUTATIONS

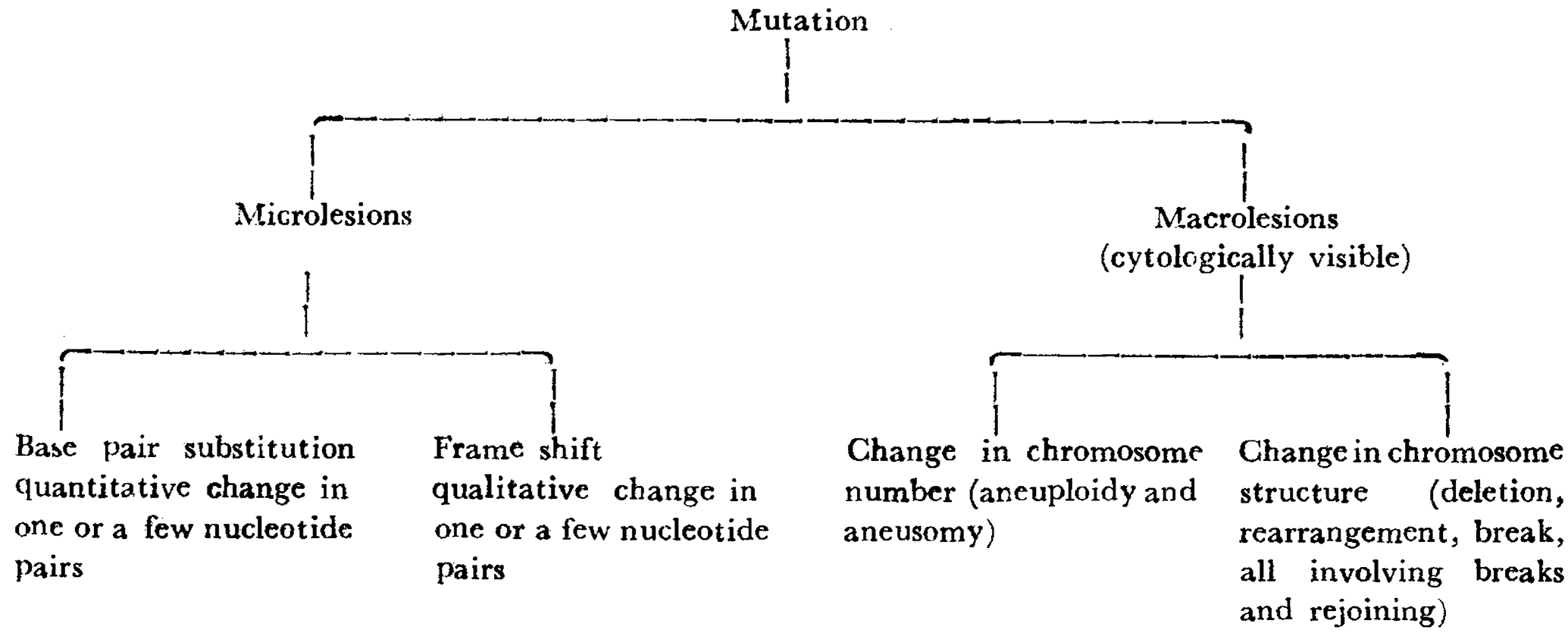

TABLE II

ACTION OF CHEMICAL MUTAGENS ON THE GENETICAL SYSTEM

I. Direct	(a) *On resting DNA* 1. Deamination of bases 2. Alkylation of bases 3. Crosslinking of DNA chains 4. Deletion and translocation of DNA molecules
	(b) *On replicating* DNA 1. Incorporation of base analogues 2. Intercalation between bases 3. Interaction with membranes 4. Interaction with enzymes
II. Indirect	1. Mistakes in DNA replicating enzymes 2. Mistakes in DNA repair enzymes 3. Events during recombination 4. Stimulation or suppression of DNA repair processes 5. Anomalous chromosome behaviour

ago. These are produced during the preparation and storage of food.

c) the more recent chemicals produced by and for modern industries, posing the greatest danger of all. Synthesis of organic substances started about 150 years ago and since then they are being continuously added to the environment.

These environmental agents may be distributed through various agencies in the soil, air and water, with the river and seas as the sink (see Sharma, 1981).

The immediate effects can be observed on the flora and the fauna and on human health. Long term effects lead to alterations in genetical components of somatic cells, with possibilities of subsequent malignant transformation and perhaps in the germ line, resulting in sterility and congenital malformations.

The major hazards are posed by the synthetic organic chemicals. Their use and manufacture have doubled almost every 7 to 8 years, the total amount being over 175 billtion pounds per year (Fishbein, 1982). Many new organic compounds have been introduced. Since these chemicals are present in products involved in human use, any toxic effects will be directly on the human system. Approximately 4.0 to 4.3 million chemicals were known to exist in 1980 (Fishbein, 1982). Their rate of numerical growth was believed to be 10% per year.

Exposure of human beings to these man-made chemicals may be (a) *direct* during the course of manufacturing a particular chemical, as in the Bhopal tragedy, or (b) *indirect*, either through using the products containing these chemicals or through long term exposure as contaminants.

A very low percentage of the approximately 1000 new chemicals introduced worldwide every year is fully tested for their mutagenicity or carcinogenicity *prior* to their release in markets. Even chemicals which are used extensively and for long periods had been manufactured for many years before they were tested for their mutagenic activity. Only about 7000 chemicals in the USA are estimated to have been checked for their carcinogenic property (Fishbein, 1979, 1982).

The interest in environmental mutagenesis has strengthened considerably following understanding of the broad overlap between mutagens and carcinogens (Higginson, 1979, Hollaender & De Serres, 1977-1980). An empirical correlation may be visualized between the mutagenic and carcinogenic properties of carcinogens (Miller & Miller, 1971). While most chemical carcinogens do not act as the ultimate carcinogenic molecules, they do yield, with metabolic transformation, intermediates with mutagenic and neoplastic activity. Based on susceptibility to the tests available, the Committee 2 of the ICPEMC (1983) has distinguished between genotoxic and non-genotoxic carcinogens.

TEST SYSTEMS

Numerous test systems, using microorganisms to laboratory mammals, both *in vivo* and *in vitro* and numerous end points, from point mutation to tumour induction, have been developed (see Sharma, 1984, Sugimura *et al.* 1982). The two major aspects were (i) testing for mutagenesis and (ii) testing for cytogenetic effects. There is considerable overlap but the methods are well-defined. In the present study, only the cytogenetic parameters, using plant as test system, are discussed.

Plant genetic systems, particularly the angiosperms, have presented unique advantages for mutagen screening, principally due to (i) the complexity of the genome, (ii) the possibility for the study of germinal and somatic changes, (iii) their exposure as *in situ* monitors and (iv) property of activating some chemical mutagens into promutagens (Ehrenberg 1971, Nilan 1978, Plewa and Gentile 1981). Some of the classical plants, widely adopted for mutagen testing, are : barley, wheat, both *Triticum aestivum* and *T. durum*, the small crucifer *Arabidopsis thaliana*, pea, maize, soybean and even rice. The methods have involved progeny testing following seed treatment with

respect to different inherited parameters, in addition to germination, sterility, pollen alterations, seedling growth and survival. The mutations can be identified on characteristics ranging from morphological, like waxy mutant in *Oryza* to biochemical tests for alterations in protein patterns (Fujii, 1982; see Survey, 1976 and Workshop Proceedings, 1978).

CYTOGENETIC TESTING

In 1930's and 1940's, the chromosomal aberrations, their mechanism, behaviour and fate, following irradiation, were studied both during mitosis and meiosis primarily on insects like *Drosophila* and grasshopper and plant systems like *Allium* and *Tradescantia*. Numerous chemicals were also shown to exert such effects, from inorganic metal salts (Van Rosen, 1957) to undistilled water (Sharma & Sen, 1954, Sharma & Sharma, 1960). However, such studies were mainly limited to academic exercises until the past decade when concern over the deteriorating environmental situation led to an increasing demand for effective short-term assay systems to screen mutagens and carcinogens. The expense and the long time involved for *in vivo* tests on animals led to tha evolution of the short term tests for both mutagens and carcinogens.

The different cytogenetic criteria fall into two categories : (a) chromosome breakage and (b) sister chromatid exchange.

CHROMOSOME DAMAGE

It is an efficient, reliable and economical criterion to measure genetic toxicity. The action of a particular chemical depends on several factors, in addition to the dosage and duration of treatment. These include, the mode of administration, the number of cycles after which the effect is observed, the interaction with other chemicals and the nature of its alteration inside the test system. The changes in the chromosome may involve alteration in structure or number or both. The agents causing chromosome break also damage the genetic apparatus and are termed *clastogens*, while those, leading to alterations in the number, mainly affect the spindle and are called *mitotic poisons*. The end effects often overlap (Table III). The manifestation of the effects of a clastogen depends on the stage of the cell cycle during which it has been applied as well (Angelosanto & Nichols, 1979). Achromatic lesions and deletions may occur following action on early synthetic and G_1 phases due to inhibition of biosynthesis of DNA. Action during late synthetic and G_2 phases may also lead to all types of chromatid aberrations, Most alkylating agents act on cells at G_1 and early synthetic phases, giving rise to chromatid aberration was well.

SISTER CHROMATID EXCHANGES

These represent the interchange of DNA strands between replication products at apparently homogenous loci. These exchanges are seen in cytological preparations of metaphase chromosomes and are used extensively to identify the carcinogenic and mutagenic effects on chromosomes. A number of known DNA damaging agents has been observed to cause significant increase in SCE frequency, typically at doses below those inducing increased chromosomal aberrations.

Use of SCE, however, suffers from the limitation that the confidence level of chromosome breakage is somewhat higher than that of SCE, specially *in vivo* and the protocols for SCE are rather cumbersome. In plants, this test is as yet not as widely employed as in mammals.

A combination of the two techniques gives much more reliable results and is

TABLE III

RELATIVE EFFICACY OF DIFFERENT TEST SYSTEMS IN MONITORING CLASTOGENS

Organism	Dominant lethality	Translocation	Deletion/ Duplication	Spindle disturbance
Algae	—	—	+	+
Fungi (Neurospora)	+	—	+	+
(Yeast)	+	—	—	+
Higher plants				
terresterial (seed and bulb)	—	+	+	+
submerged	—	—	+	+
Insects				
Drosophila	+	+	+	+
Habrobracon	+	+	—	—
Fish				
Tilapia	—	+	+	+
Mammalian				
cell culture	—	+	+	+
Mammals *in vivo*				
Mus and Rattus	+	+	+	+
Man	—	+	+	+
Avian embryo	—	+	+	+

able to detect clastogenicity at any level. Detailed calculations of chromosome breakage are needed only when in-depth studies are required. For a routine drug assay, it is only needed to know if a drug can cause chromosomal damage and if so, to what extent (Hsu, 1982). For obtaining quantitative data, chromatid aberrations, which involve break in the G_2 phase, are more suitable than chromosome aberrations. In any case, study of chromosome aberrations should be routine in measuring genetic toxicity, with or without confirmation from SCE data.

MICRONUCLEUS TEST

It was developed for the detection of freshly induced structural chromosomal alterations in the bone marrow of animals *in vivo* (see Sharma, 1984). Micronuclei originate from chromatin which lags during anaphase, indicating spindle distur-

bance. Though originally located in erythroblasts, yet this method is applicable to plant systems as well.

Before mammalian cells could be sudied *in vitro* most of the information on the effects of mutagenic chemicals on chromosomes was obtained from plant materials, from microspores of *Tradescantia* to the bulbs of *Allium cepa* (Hollaender, 1971-76; Sharma & Sharma, 1960; Kihlman, 1971). The meristematic cells were used, usually from the root tip or shoot tip and also stamen hair as in *Tradescantia*. *Vicia faba* (2n=12) is a very popular material principally due to the large chromosome size, low number of chromosomes, identifiable heterochromatin blocks (Plewa, 1982) and sensitivity to SCE tests (Kihlman & Kronberg, 1975). Structurally reconstructed karyotypes of *Vicia faba* are able to indicate chromosome breaks more precisely than the standard ones. These special types were raised by systematic crossing of plants with single translocations or inversions, so that multiple reconstructed karyotypes in which all six chromosome pairs can be easily identified are obtained ((Michaelis & Rieger, 1971).

Mutagenicity in plants is of primary importance since the initial effect of environmental agents is usually on plant ecosystems. The conversion of relatively less toxic inorganic chemicals into organic forms is also only possible through plants. However, as test systems, the use of higher plants has certain limitations, if the ultimate target is man, due to differences in physiology, metabolism and difficulty in interpolating the information to the human system. Nevertheless, response to a number of chemicals of plant chromosomes is found to be comparable to mammalian cells *in vitro* and *in vivo*, *Drosophila* and even bacteria (Nilan, 1978; Table III).

Extrapolation of the data obtained from different test systems to human risk assessment presents a major problem since the final goal for mutagenicity testing is the human system. The relative characters of some test systems as applied to man are given in Table IV (after Anderson & Ramel, 1979).

Inspite of the very large number of test systems at present available and being added to, higher plants still retain their importance in identifying the harmful effects of chemicals both at genic and chromosomal levels. To fully annalyse the genotoxic potential of any agent, a battery of multiple tests is required. However, the results are not necessarily identical in lower and higher organisms, with respect to application to man, the ultimate target. The expense and time involved in testing all possible combinations of period of treatment and dosages on mammals, *in vivo* and *in vitro*, make it a formidable task, which is not often undertaken by the manufacturers. A classical case is of the thalidomide mishap, leading to armless and legless babies, following the ingestion of the drug by the mothers during pregnancy. Later information has revealed that less than half a dozen rabbits had been used in the experiments.

The higher plants may thus form a screening system for all new chemicals, to obtain the upper and lower threshold limits, for chronic exposure at subtoxic doses. Variations at the cytogenetic levels indicate potential clastogenicity and turbagenicity. Later experiments on higher mammals may therefore be limited within the range identified through such experiments.

In addition to the application to human damage, cytogenetic effects on plants systems also indicate the tolerance range of the chemicals, on prolonged exposure. This information can be utilised in assessing the upper limit of discharge of parti-

TABLE IV

RELATIVE CHARACTERS OF SOME TEST SYSTEMS AS APPLIED TO MAN

Test system	Time taken	Relative ease of detecting	
		Gene mutations	Chromosomal aberrations
Microorganisms with metabolic activation			
Salmonella typhimurium	2 to 3 days	Excellent	—
Escherichia coli	2 to 3 days	Excellent	—
Yeasts	3 to 5 days	Good	Unknown
Neurospora crassa	1 to 3 weeks	Very good	Good
Cultured mammalian cells with metabolic activation	2 to 3 weeks	Excellent to fair	Unknown
Host-mediated assay with			
Microorganisms	2 to 7 days	Good	—
Mammalian cells	2 to 5 weeks	Unknown	Good
Body fluid analysis	2 days	Excellent	—
Plants	—	—	Good
Vicia faba	3 to 8 days	—	Relevance to man not clear
Tradescantia paludosa	2 to 5 weeks	Good	
Vallisneria spiralis	2 to 5 weeks	potential	—
Allium species	One to 30 days	—	
Insects			
Drosophila melanogaster	2 to 7 weeks	Good to very good	—
Mammals			
Dominant lethal mutations	2 to 4 months	—	Unknown
Translocations	5 to 7 months	—	Potentially
Blood or bone marrow cytogenetics	1 to 5 weeks	—	Potentially very good
Specific locus mutations	2 to 3 months	Unknown	—

cular chemicals in the atmosphere, without genotoxic after-effects, while formulating containment measures.

REFERENCES

ANDERSON, D. AND C. RAMEL 1979. In : *Genetic damage in man caused by environmental agents*. ed. K. Berg, Academic Press, New York.

ANGELOSANTO, A. AND W. W. NICHOLS 1979. *Mutat. Res.* **65 :** 133.

EHRENBERG, L. 1971. In : *Chemical Mutagens*. ed. Hollaender, A. **2 :** 365-386, Pleum Press, New York.

FISHBEIN, L. 1979. *Potential industrial mutagens and carcinogens*, Elsevier, Amsterdam, p. 534.

FISHBEIN, L. 1982. In : *Environmental mutagens and carcinogens*, ed. Sugimura, T., Kondo, S. and Takebe, H., University of Tokyo Press, Tokyo and Alan R Liss Inc., New York, p. 21.

FUJII, T. 1982. In : *Environmental mutagens and carcinogens*, ed. Sugimura *et al.*, University of Tokyo Press, Tokyo pp. 399-410.

HIGGINSON, J. 1979. In : *Environmental carcinogenesis, occurrence, risk evaluation and mechanisms*. ed. Emmslot, P. and Krick, E. Elsevier, North Holland, Amsterdam.

HOLLAENDER, A. 1971-1976. ed. *Chemical Mutagens* **1—6,** Plenum Press, New York.

HOLLAENDER, A. AND F. J. DE SERRES 1977-1980. ed. *Chemical mutagens : principles and methods for their detection*, **1—6,** Plenum Press, New York.

HSU, T. C. 1982. ed. *Cyrogenetic assays of environmental mutagens*. Allenheld, Osmun, Totowa, New Jersey.

ICPEMC—Commiittee 2 final report 1983. *Mutat. Res.* **114 :** 117.

KIHLMAN, B. A. 1971. In : *Chemical mutagens* **2 :** 489, ed. Hollaender A, Plenum Press, New York.

KIHLMAN, B. A. AND D. KRONBERG 1975. *Chromosoma* **51 :** 1.

MICHAELIS, A. AND R. RIEGER 1971. *Chromosoma* **35 :** 1.

MILLER, J. A. AND E. C. MILLER 1971. *J. natl. cancer Ins*. **47 :** and *IV*.

NILAN, R. A. 1978. *Environmental Hlth. Persp.* **27 :** 181-196.

PLEWA, M. J. 1982. In : *Environmental Mutagens and carcinogens*, ed. Sugimura *et al.*, University of Tokyo Press, Tokyo, 411-419.

PLEWA, M. J. AND J. M. GENTILE 1981. In : *Chemical Mutagens*. ed. F. J. De Serres **7 :** 401-420.

SHARMA, A. 1984. Environmental chemical mutagenesis, *Persp. Rep. Ser.* **6 :** Golden Jubilee Publications, Indian Nation. Sci. Acad. New Delhi, 1-53.

SHARMA, A. K. 1981. Presidential Address during the 68th Session of the Indian Sci. Congr. Assoc. Varanasi, on *Impact of the development of Science and Technology on Environment*. ISCA Publ., Calcutta.

SHARMA, A. K. AND S. SEN 1954. *Genet. Iber.* **6 :** 19.

SHARMA, A. K. AND A. SHARMA 1960. *Int. Rev. Cytol.* **10 :** 101.

SUGIMURA, T., S. KONDO AND TAKEBE, H. 1982. (ed) *Environmental mutagens and carcinogens*, University of Tokyo Press, Tokyo and Alan R. Liss Inc., New York.

VON ROSEN, H. 1957. *Hereditas* **43 :** 644.

SURVEY OF LITERATURE ON CHEMICAL MUTAGENESIS IN PLANTS AND MUTAGENESIS OF PLANT RELATED COMPOUNDS 1976. *Environmental Mutagen Information*, Oak Ridge National Laboratory, Tennessee, USA.

WORKSHOP ON HIGHER PLANTS AS MONITORS OF ENVIRONMENTAL MUTAGENS 1978. Florida, USA.