Environmental Health Perspectives
Vol. 27, pp. 181–196, 1978

Potential of Plant Genetic Systems for Monitoring and Screening Mutagens

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Plants have too long been ignored as useful screening and monitoring systems of environmental mutagens. However, there are about a dozen reliable, some even unique, plant genetic systems that can increase the scope and effectiveness of chemical and physical mutagen screening and monitoring procedures. Some of these should be included in the Tier II tests. Moreover, plants are the only systems now in use as monitors of genetic effects caused by polluted atmosphere and water and by pesticides.

There are several major advantages of the plant test systems which relate to their reproductive nature, easy culture and growth habits that should be considered in mutagen screening and monitoring. In addition to these advantages, the major plant test systems exhibit numerous genetic and chromosome changes for determining the effects of mutagens. Some of these have not yet been detected in other nonmammalian and mammalian test systems, but probably occur in the human organism.

Plants have played major roles in various aspects of mutagenesis research, primarily in mutagen screening (detection and verification of mutagenic activity), mutagen monitoring, and determining mutagen effects and mechanisms of mutagen action. They have played lesser roles in quantification of mutagenic activity and understanding the nature of induced mutations.

Mutagen monitoring with plants, especially *in situ* on land or in water, will help determine potential genetic hazards of air and water pollutants and protect the genetic purity of crop plants and the purity of the food supply. The *Tradescantia* stamen-hair system is used in a mobile laboratory for determining the genetic effects of industrial and automobile pollution in a number of sites in the U.S.A. The fern is employed for monitoring genetic effects of water pollution in the Eastern states. The maize pollen system and certain weeds have monitored genetic effects of pesticides. Several other systems that have considerable value and should be developed and more widely used in mutagen monitoring and screening, especially for *in situ* monitoring, are discussed. Emphasis is placed on pollen systems in which changes in pollen structure, chemistry, and chromosomes can be scored for monitoring; and screening systems which can record low levels of genetic effects as well as provide information on the nature of induced mutations.

The value of plant systems for monitoring and screening mutagens can be improved by: greater knowledge of plant cell processes at the molecular and ultrastructural levels; relating these processes to mutagen effects and plant cell responses; improving current systems for increased sensitivity, ease of detecting genetic and chromosome changes, recording of data (including automation), and for extending the range of genetic and chromosome end points; and designing and developing new systems with the aid of previous and current botanical and genetic knowledge.

Introduction

Although not generally recognized, there are several plant genetic systems which possess the characteristics for screening and monitoring environmental mutagens. They are sensitive test systems and have already provided reliable and useful quantitative mutagenesis data (*1, 2*). These systems have played initial roles in detecting new mutagens, advancing the knowledge of mechanisms of action of certain mutagens, and developing techniques that were later used in other systems for advancing mutagenesis knowledge. In the area of mutagen monitoring there are no other eukaryotic or prokaryotic systems as useful at the present time as *Tradescantia* stamen-hairs for air pollution (*3, 4*) and the fern for water pollution (*5–8*).

Unfortunately, most of the major activities of mutagenesis research in plant systems have been overlooked and often excluded, particularly when aims of the research are to learn more about mutagenesis in man. For instance, the recent DHEW report on chemical mutagen screening (*9*), which surveyed numerous test systems for establishing those most relevant for mutagen screening and

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monitoring, included only *Tradescantia* stamen-hairs for screening plant mutations. The Environmental Mutagenesis Society Committee 17 report (*10*), having similar objectives, cites only chromosome aberrations in *Vicia* and *Tradescantia* and the stamen-hair system in *Tradescantia* among recognized plant test systems. There appeared to be no botanists or plant geneticists on the committees preparing these reports. This holds true for other reports and proceedings of major conferences that are establishing batteries of tests and tier or screening systems.

The lack of general recognition of plant test systems no doubt stems in part from the perception that plant and animal, especially human, cells appear to be so distantly separated physiologically and phylogenetically to make mutagenesis data gathered from plant cells of little relevance. Clearly, in major mutagenesis circles, higher plant cells, because of cell walls, different metabolism, etc., are considered to have little value for gathering data that can be extrapolated to man as contrasted to mammalian systems and even such nonmammalian systems as *Drosophila, Neurospora,* yeast, and bacteria.

Another reason for the lack of recognition of plant test systems in mutagen screening and monitoring may well be the relatively high proportion of inconclusive and irrelevant research, particularly in the area of chemical mutagenesis, that has been conducted with plants throughout the past 15 to 20 years. Many papers utilizing plant genetic systems present little or no quantitative data or least effective doses of mutagens, and thus provide no information about the mechanism of mutagen action and no basis for valid comparisons with data from other systems. Certainly many plant mutagenesis papers do not contain the experimental precision, the statistical analyses, and data of general value of many papers utilizing mammalian and certain nonmammalian systems. Thus, they attract little or no attention from the mammalian and microbial geneticists who are spearheading most of the mutagenesis research in the world. Rather, much of the published research has been devoted to elucidating merely the effects of mutagens and the production of mutants for plant improvement programs, genetic studies, etc.

Another problem in plant mutagenesis research is that much good published information in plant mutagenesis has not been utilized in developing new knowledge and technology, and useful monitoring and screening plant test systems.

Another reason for the neglect of plant test systems for mutagen screening and monitoring is poor "public relations." Fellow scientists and influential personnel of government agencies do not know about the good plant mutagenesis research and the potential of plants as mutagen test systems. Thus, lack of promotion of the positive aspects of plant mutagenesis research and its relatively poor research image has led to low funding priority in national agencies and a decline in good research productivity. The number of scientists in plant mutagenesis in the U.S.A. has decreased over the past several years.

Fortunately, several plant systems were included in the recent international Comparative Chemical Mutagenesis Workshop (*11*) organized and developed by Dr. F. J. de Serres and his colleagues of NIEHS. In this Workshop, about 25 test systems, from bacteria to human cell culture, were analyzed for responses to 20 real or putative chemical mutagens and carcinogens. This Workshop provided an interaction among scientists working on mammalian, plant, and other nonmammalian systems, and provided an opportunity for the plant scientists to promote the advantages of plant test systems to scientists working with other test systems and in government agencies concerned with testing for environmental mutagens and carcinogens.

Moreover, representatives from just about every laboratory in the U.S.A. and the only laboratory in Canada working on plant mutagenesis and plant test systems attended the Workshop on Higher Plant Systems as Monitors of Environmental Mutagens. This was the first time many of the representatives met and interacted. Such interactions will hasten progress in relevant areas of plant mutagenesis research.

Plant genetic systems have several unique advantages for mutagen screening and therefore should be included in the tier screening system. They at present provide the only currently used monitors of air and water pollution. Through more diligent work with the plant genetic systems now available and by exploiting potentially valuable systems still little used in mutagenesis research, plants can become even more useful for monitoring and screening environmental mutagens. This paper will review, without too much repetition of the information presented in other papers of this workshop, the current scope and potential of plant test systems for mutagenesis research, especially mutagen screening and monitoring.

Some Characteristics of Plant Cells That May Affect Mutagen Response

In any assessment of the role of plants as test systems for screening and monitoring environmen-

tal mutagens there first must be an understanding of plant cell structure and processes. For greater recognition of plant cells in mutagenesis research this assessment must lead to a comparison of these cells with animal cells and an identification of differences that might lead to differential mutagen effects.

In general, the knowledge in plant molecular biology is so relatively scant that many of the more important processes and structures are little understood—thus making any comparison with the somewhat more detailed knowledge in animal cells somewhat meaningless. However, it is important in a workshop such as this to indicate at least some processes and structures which could be important in mutagen absorption and action, and in mutant survival and detection. Some of these have been described (*12, 13*), and some will be presented in more detail in these proceedings (*14*).

Scientists in plant mutagenesis should urge more work in plant molecular biology so that interpretations of and conclusions from mutagenesis results from plants will be more meaningful and more useful for comparisons with mutagenesis results from mammalian cells. The lack of knowledge about basic plant processes should be corrected in the near future as more Federal research money is applied to a number of basic problems in plant molecular biology and genetics.

As described in most current textbooks on cell biology, there are a number of well-known gross differences in anatomy and physiology between plant and animal cells. These may affect how the different cells react to mutagens. Eventually more detailed analyses of the differing structures and processes may show still greater differences or, on the other hand, greater similarities between plant and animal cells at the level of organization that affects mutagen action and mutant detection. Thus, it is not fruitful at this time to speculate on how these structures and physiological differences may relate to differential mutagen responses.

Of course, one of the major differences between animal and plant cells is the rigid cellulose wall of the plant cell which might account for considerable differences in mutagen uptake. The basic chemistry and structure of the plant cell wall is being developed and is described in more detail by Heslop-Harrison (*14*). Often the plant cell wall is viewed as a rigid nonpermeable plate which does not permit inward flow of certain chemical substances which readily flow into animal cells. However, these walls do have plasmodesmata through which general intercommunication and ready exchange of materials among neighboring cells occur.

Concerning cell and nuclear membranes, it is obvious that there is not enough knowledge about plant cells to make any meaningful analyses or comparisons. Certainly plant and animal cells differ in the kinds of compounds they absorb and exchange, and these differences may reflect at least some differences in structure and chemistry of their membranes.

Most mature plant cells possess a large central vacuole, while in animal cells vacuoles are small and frequently numerous. The large vacuole pushes the cytoplasm of the plant toward the outer edges of the cell where a ready exchange of gases can take place.

There are fewer mitochondria in green plants than animal cells. This is probably because their function in plants is taken over by chloroplasts. Mitochondrial DNA constitutes a second genetic site in terms of mutagen action in both plants and animals (*15*). It must be noted that there is much more DNA in plant than in animal mitochondria.

Plastids, usually chloroplasts, which characterize many of the cells of green plants, are not found in animal cells. The DNA of the chloroplast represents a third genetic site for mutagen action not found in animals (*15*). Its function and structure is now being studied intensively. Information from studies of mitochondria and chloroplast DNA should broaden the scope of plant mutagenesis and especially the induction of cytoplasmically inherited traits.

The gross features of chromosome behavior appear similar, although plants do not have centrioles as do animal cells (*16*). It is true that among plants different patterns of meiosis and gametogenesis occur, and these in turn are different than those in animal cells (*16*). An understanding of basic processes of chromosome pairing and crossing over in eukaryotes has come from research on lily (*17, 18*). It may be assumed that similar basic mechanisms of crossing over occur in animal cells. The synaptinemal complex, which affects chromosome pairing and may be involved in crossing over, may be implicated in effects of mutagens on chromosome behavior, crossing over, and nondisjunction. Apparently its structure and chemistry is generally similar in plant and animal cells (*18–20*).

In cell division, the separation of the cytoplasm in plants is accomplished by the formation of a cell plate whereas in animal cells the cytoplasm is divided by a constriction. More details of this need to be known in order to understand the effect of this major difference on mutagenic action.

At the molecular level there are apparently certain similarities and differences in processes in plant and animal cells. How these different processes may produce differential mutagen response is not known. DNA of plant and animal cells appears to be similar in structure and function. However, all plant

DNA examined to date contains about 5–10% of 5-methyl cytosine which is not found in bacteria, fungi, chloroplast and mitochondrial DNA. It occurs to a considerably lesser extent in animal DNA (*21*).

The recently discovered satellite DNA's exhibit no differing uniform patterns between plant and animal cells. Indeed, the variations in these DNA's within the plant or animal kingdoms are quite great (*22*). A recent report has identified some of the variations in plant satellite DNA's (*23*).

At the limit of our knowledge about plants, protein synthesis appears to be similar in plant and animal cells. For instance, the wheat germ translation system is used to read purified messages in humans, bacteria, and yeast as well as plants.

In metabolic activity some specific differences may now be identified. Enzyme systems and microbodies apparently differ. Plants also have glyoxysomes which are not found in animal cells. A quite basic difference between plant and animal cells has been found by Rich and Bendall (*24*) in the electron transport system. They report that the electron transport components of mammalian microsomes are well characterized and that only two cytochromes, b_5 and P-450, are generally present. The function of the latter is known but the function of the former is still unclear. Much less is known of the electron transport component of plant microsomes. Rich and Bendall found the two cytochromes in a wide variety of plant microsomes. Moreover, they uncovered some previously undescribed cytochromes not found in animal cells. Here again, the significance of these differences for mutagenesis is not known but may relate to differential metabolic activation of certain chemicals into mutagens.

The differences between plant and animal cells in terms of their hormones are vast and may be significant in mutagenesis. There are five groups of plant hormones which are generally chemically distinct from the several dozen animal hormones. It also appears that more major plant activities are under hormonal control than animal activities, especially in early development.

Mutagen Response of Plant and Other Test Systems

Although there are major differences in gross anatomy and physiology, and minor differences in several basic features of plant and animal cells, it may be significant that the response to mutagens of plant, other nonmammalian, and mammalian test systems obey similar basic rules. This was illustrated in part by recent studies in which plants, animals, and bacteria were measured in terms of their responses, especially to x-rays (*25*) and ethyl methanesulfonate (EMS) (*26*) on a mutation rate per DNA content/genome basis. In these analyses, plant cells responded in a predictable manner, and this response was similar to those of mammalian cells, *Drosophila*, and bacteria—major screening systems. The validity of these interspecific comparisons of x-ray and EMS-induced mutation rates, however, has been seriously questioned (*27, 28*).

More recent information in the comparison of mutagen responses of plant and animal cells has been provided by Clive and Spector (*29*). They screened all of the Group I reports from the Workshop on Comparative Chemical Mutagenesis comparing the mutagenicity of 20 chemicals over all systems and mutagens involved, and ranking the various systems in terms of mutagenic potency of each mutagen tested. Mutagen potency in this study is the ratio of mutation frequency/dose with a high rank indicating high potency and a low rank indicating low mutagen potency. In this ranking, plant test systems fare favorably in determining mutagen potencies compared to other nonmammalian systems, *in vivo* and *in vitro* mammalian systems, and bacteria.

There were eight chemicals for which there were reasonable rankings of mutagen potency for most of the plant systems (barley, soybean, onion, *Vicia*, and *Tradescantia*), the *in vitro* and *in vivo* mammalian systems, bacteria, and *Drosophila*. In other words, there was sufficient information over all systems for eight of these chemicals where comparisons have been meaningful. The chemicals were ethyl methanesulfonate (EMS), methyl methanesulfonate (MMS), ethyleneimine (EI), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), dimethylnitrosamine (DMN), triethylenemelamine (TEM), trenimon (TREN), and mitomycin C (MMC).

In Table 1 these rankings show that the plant systems responses to the eight chemicals are quite similar to those of the *in vitro* mammalian systems.

Table 1. Ranking of mutagenic potencies (frequency/dose) of eight chemical mutagens for plant and other test systems.[a]

Plants,	Mammals			
in vivo	*In vivo*	*In vitro*	Drosophila	Bacteria
TREN	TEM	TREN	TEM	MMC
MMC	TREN	MMC	TREN	TEM
MNNG	MMC	EI	MMS	EI
TEM	EI	MNNG	DMN	DMN
EI	DMN	TEM	MNNG	MNNG
EMS	MNNG	MMS	EMS	MMS
MMS	MMS	DMN	MMC	TREN
DMN	EMS	EMS	EI	EMS

[a] Data of Clive and Spector (*29*).

However, plant responses are more similar to the two mammalian systems than those of *Drosophila* and bacteria. For instance, observing the rankings of the eight chemicals for plants and *in vitro* mammalians, it is obvious that EMS, MMS, and DMN rank near the bottom, while TREN, MMC, and MNNG are in the upper group in terms of mutagen potency. In *Drosophila*, MMS and DMN are in the upper group while MMC and MNNG are in the lower group. Bacteria also show some discrepancies when compared to *in vitro* and *in vivo* mammals.

It must be recognized that these rankings at the best can only be suggestive. Nevertheless, they do indicate that plant systems responses to specific mutagens compare favorably to mammalian systems as well as other nonmammalian test systems.

Grant (*30*) has summarized a number of studies on pesticide effects which show that there is an excellent correlation between frequencies of chromosome abnormalities as scored in plants and mammalian cell systems. There is also a good correlation for mutagenic activity. Thus, it would appear that plants can be as valuable for mutagen screening as some of the widely used mammalian and nonmammalian test systems.

Advantages of Plant Test Systems

Plants have numerous advantages for mutagenesis research (*1, 2, 30, 31*). Some of these are summarized here: (1) They are easy to culture and some permit regeneration from cells. (2) Several provide chimeral situations not found in animals and cells from chimeras can be regenerated. (3) The chromosome organization of plants is similar to that of human and mammalian cells. (4) Some have short generation times. (5) The cost of culturing, the cost and time of training technicians to handle a variety of end points following mutagen treatment and space requirements are relatively small. (6) Several yield relatively good genetic information. (7) Particularly when seeds are used, the mutagenic effects can be studied under a wide range of environmental conditions, such as large differences in pH, water content, temperature, and metabolism. (8) Certain of the test systems permit detection of mutants within days after treatment. (9) Several plant test systems provide unique advantages for *in situ* monitoring and measuring of responses to chronic treatments. (10) A wide range of genetic end points can be scored following mutagen treatment.

Genetic End Points

A major advantage of plant test systems is the numerous genetic and chromosome changes that can be scored following mutagen treatment. Some of these changes that can be scored in the major plant test systems are listed in Table 2. Some of the better plant systems are quite mutagen-sensitive and exhibit genetic and chromosome alterations which have been detected in the widely used mammalian test systems, following physical or chemical mutagen treatments. Moreover, certain plants permit the analysis of several genetic end points. Furthermore, events which have been detected in plants, such as somatic crossing over, have not yet been identified or utilized as mutagen test systems in well-studied mammalian systems.

Other papers in this workshop have described some of these genetic end points in detail. The purpose here is to summarize them and indicate details not described elsewhere.

Mutations. Mutation frequencies are usually reported for most genetic systems used in plants. However, for some plant systems, e.g., barley, it is possible to determine mutation rates per locus. The latter permits a more realistic comparison of the response of plant systems and other test systems following treatment with a given mutagen.

MULTIPLE LOCUS SYSTEMS: Barley, *Arabidopsis*, maize, and tomato, in which chlorophyll-deficient mutations are scored, provide very sensitive test systems in that a variety of genetic events at a large number of loci are measured. These multilocus systems can be likened to the recessive-lethal system of *Drosophila* which is considered one of the most sensitive eukaryotic systems for measuring mutagen activity. In *Drosophila*, mutations at about 800 loci are monitored, and these mutations apparently range from point mutations in DNA to deletions and chromosome rearrangements (*32*). The same is true for the plant systems. For instance, in barley it has been recently estimated (*33*) that probably 700 to 800 loci are involved in chlorophyll development and a mutation at any one of these loci will produce a chlorophyll-deficient seedling mutant. Here too, a range of mutational events is occurring. Multiple locus systems such as those in barley, *Arabidopsis*, and *Drosophila* are considered more relevant than single locus systems for predicting mutagen response in man.

SPECIFIC LOCUS SYSTEMS: Specific locus systems are also available for mutagen screening and monitoring in plants. Several are well developed and quite sensitive (*31*). The specific locus systems include the $Y_{11}y_{11}$ locus in soybean (*34*), the Yg_2yg_2

Table 2. Plant test systems and genetic end points most widely used in mutation research and mutagen screening.

Plant	Genetic end points
Tradescantia (spiderwort, $2n = 24$)	Mutations in stamen hair cells
	Chromosome changes at mitosis
Vicia faba (broadbean, $2n = 12$)	Chromosome changes at mitosis, chromosomes morphologically distinct
Crepis capillaris (hawksbeard, $2n = 6$)	Chromosome changes at mitosis, chromosomes morphologically distinct
Glycine max (soybean, $2n = 40$)	Somatic crossing over at specific locus ($Y_{55}y_{11}$)
	Mutations at specific locus ($Y_{11}y_{11}$)
Hordeum vulgare (barley, $2n = 14$)	Mutations at multiple loci, chlorophyll development
	Mutations at specific loci, *waxy* pollen, chlorophyll loci
	Chromosome alterations at mitosis and meiosis
	Micronuclei in meiotic tetrads
	Embryo lethals
	Single strand DNA breaks
	Aneuploidy
Zea mays (maize, $2n = 20$)	Mutations at multiple loci, chlorophyll development
	Mutations at specific loci, *Adh* and *waxy* pollen, yg_2
	Chromosome changes at meiosis
	Embryo lethals
	Aneuploidy
Arabidopsis thaliana (mouse ear cress, $2n = 10$)	Mutations at multiple loci, chlorophyll development
	Mutations at specific locus, thiamine production
	Embryo lethals
Pisum sativum (pea, $2n = 14$)	Mutations at multiple loci, chlorophyll development
	Mutations at specific loci
Allium cepa (onion, $2n = 16$)	Changes in mitotic chromosomes
Lycopersicon esculetum (tomato, $2n = 16$)	Mutations at multiple loci, chlorophyll development
	Mutations at specific loci
	Chromosome alterations at meiosis
	Aneuploidy

locus in maize (*35, 36*), and certain chlorophyll loci in barley (*37, 38*). Those involving male gametophytes or pollen can detect mutation events at extremely low frequencies (*39*). They include S (self incompatibility) loci in several plants (*40, 41*), *waxy* locus (pollen and endosperm) in maize and barley (*42, 43*), and the alcohol dehydrogenase locus (pollen) in maize (*44, 45*).

CYTOPLASMIC MUTATIONS: Cytoplasmic mutations, studied much more extensively in plants than in animals, must be considered in the total response of humans to mutagens. They provide another dimension for mutagen testing and monitoring, and most of them can be attributed to changes in the DNA of the chloroplast and of the mitochondria (*15, 46*).

CONTROLLING ELEMENTS: Mutations induced by controlling elements have been studied much more extensively in plants than in animals and present an additional dimension for analyzing mutagen effects. They have been investigated most thoroughly in maize, but have also been detected in *Antirrhinum majus, Impatiens balsamina,* and tobacco. The nature, behavior, and variations of these controlling elements in plants have been summarized (*47*), and it has been recently suggested that the position of the controlling element at the locus determines the state of mutability (*48*). Analogies between the properties of controlling elements and those of episomes and plasmids in bacteria have been pointed out frequently (*49*).

PARAMUTATION: Paramutation is another mutational event in plants which has not been detected in animals. It involves a high-frequency directed heritable change at a locus which leads to new alleles as a result of allelic interaction. The nature and properties of this phenomenon in maize and other plant species have been reviewed (*50*).

Studies of controlling elements, paramutation, and other similar phenomenon, which lead to new alleles, suggest that a variety of events involving apparent regulatory mechanisms occur and can be induced. They also reveal the very great complexity of the eukaryotic genome and of the chromosome processes that lead to expression of structural genes and that are involved in mutation.

Single-Strand DNA Breaks. Alkylating agents such as MMS, EMS, dES (diethyl sulfate), MNU (*N*-methyl-*N*-nitrosourethane), and iPMS (isopropyl methanesulfonate), induce single-strand breaks in DNA of barley embryonic cells (*51*) and the shape of the dose response curves resembles those obtained for chromosome aberrations (Veleminský and Gichner, unpublished). Dose dependent induction of DNA single-strand breaks by gamma rays was observed in barley (*52*), and in carrot protoplasts (*53*).

DNA Repair. In plants, repair of chemical in-

duced DNA lesions has been most extensively studied in treated barley embryos stored under a range of temperature and moisture conditions (*54, 55*).

The repair takes place before the onset of DNA synthesis as this synthesis starts after the stored seeds are sown and germinate. The endonuclease specific for apurinic sites in DNA was isolated from barley embryos and leaves. This endonuclease is most probably responsible for the rapid formation of DNA breaks from apurinic sites induced by alkylating agents and takes part in the observed repair pathway (*51, 54*). Another step of this process represents the repair synthesis detected in barley by means of ^{3}H BUdR and isopycnic CsCl gradient centrifugation in seeds and embryos treated with methyl nitrosourea (*54, 55*).

Excisionlike repair of DNA single-strand breaks was observed during the posttreatment washing of seeds, during submerged storage of treated seeds under anaerobic conditions, and in seeds treated with the mutagen and sodium azide (*51, 54, 55*). In all three cases, however, methyl nitrosourea was used as a mutagen.

Repair of radiation-induced DNA breaks has been observed in carrot protoplasts (*53*). The prereplication repair of γ-ray-induced DNA lesions was detected by unscheduled DNA synthesis observed autoradiographically during the early stage of germination of γ-irradiated seeds (*56*) and by differential activity of DNA polymerase in γ-irradiated and nonirradiated samples as well as in radiosensitive and resistant varieties (*57*).

Chromosome Changes. CHROMOSOME AND CHROMATID BREAKS: Plants have long been noted and utilized extensively for screening mutagens in terms of their clastogenicity (ability to break chromosomes and chromatids). Many of the well-studied plants such as *Vicia faba* ($2n = 12$), *Tradescantia* ($2n = 24$), barley ($2n = 14$), *Crepis capillaris* ($2n = 6$), onion ($2n = 16$), and lily ($2n = 24$), possess chromosomes that are large and relatively few in number. Some, such as *Vicia faba (58, 59)* and *Crepis capillaris (60, 61)* possess morphologically distinct chromosomes. Indeed, *Crepis* is one of the most widely used plant test systems for mutagenesis research in the Soviet Union (*62*). *Haplopappus,* with a diploid chromosome number of 4, should be used more extensively in screening for clastogenicity. New knowledge about chromosome breakage and aberrated chromosome behavior in radiation and chemical mutagenesis has evolved from appropriate experiments with these plants.

Reconstructed karyotypes such as recently developed in *Vicia* (*63*) and barley (*64*), C banding capability in *Vicia faba* (*65*), barley (*66*), lotus (*67*), and lily (*68*), and Q banding in lily (*68*), add to the resolution of chromosome aberration analyses. They help to more conclusively determine the origin and distribution of chromosome breaks, to detect differential chromosome breakage among chromosome arms, and identify minute chromosome rearrangements which could not be detected in normal karyotypes and without banding markers.

SUBCHROMATID ABERRATIONS: Subchromatid aberrations have been detected in plants (*69*) and used for screening effects of chemicals (*70*).

SOMATIC CROSSING OVER: Somatic crossing over leading to somatic recombination is a unique genetic end point, readily detectable in plants and already utilized extensively for mutagen screening. This end point, which is not readily detected in animal systems, has been most utilized in soybean (*34, 71*) and to a lesser extent in tobacco (*72, 73*).

NONDISJUNCTION: Nondisjunction which leads to aneuploidy, a most important chromosome aberration plaguing the human organism, is easily detected and analyzed cytologically as well as genetically in plants (*74*). In genetically well studied species such as maize, barley, tomato, wheat, tobacco, and *Datura,* aneuploids have been intensively investigated. In these plants, trisomic and/or monosomic lines which have originated from nondisjunction have been established for each chromosome, and have been maintained with high fertility for gene mapping and other genetic uses. Multicentromeric species such as *Luzula* (*75, 76*) should be useful for adding to the knowledge of nondisjunction.

SISTER-CHROMATID EXCHANGE: Sister-chromatid exchanges are now considered to be a very sensitive measure of mutagen damage in mammalian systems. Although their origin and biological significance are not yet known, considerable emphasis has been put on their use as detectors of mutagen activity. Techniques for determining sister-chromatid exchanges in plants have recently been initiated. Already this technique is well developed and utilized in mutagen screening in *Vicia faba* (*77, 78*).

MICRONUCLEI: The micronucleus test, used for mutagen screening in mammalian systems, can be easily developed in several plants, e.g., barley and *Tradescantia.* However, its use has been negligible to date in mutagen screening in plants.

CHIASMATA: Chiasmata can be easily seen in several plant species (*16*), thus allowing measurements of a given mutagen to increase or decrease chiasmata frequency.

Male Sterility and Embryo Lethals. Male sterility and embryo lethals are easily measured traits in plants, and increases in these end points

can be measured as a result of mutagen treatment. They are a somewhat crude measure of gene and chromosome changes.

Multi-End Points. Several plants permit the analyses of more than one genetic end point in determining the response to a given mutagenic treatment. This capability broadens the bases for interpreting mutagen action. For instance, in barley, both chromosome aberrations and mutations can be detected following a given treatment. Thus it is possible to compare the effectiveness of different mutagens for inducing both end points. X-rays produce a low ratio of mutations to chromosome breaks; ethyleneimine produces a higher ratio; ethyl methanesulfonate a still higher ratio; and finally, sodium azide which induces a very high frequency of mutations but relatively few if any chromosome aberrations.

Additional end points can be detected in barley. In fact, from a single mutagen treatment of barley seed the following end points can be scored: frequencies of mutations at a specific locus and at multiple loci; frequencies of a variety of chromosome aberrations during mitosis and meiosis; frequencies of micronuclei in meiotic tetrads; amounts of sterility; frequencies of embryo lethals; and frequencies of single-strand DNA breaks. Such an array of end points permits considerable insight into the effects and action of a given mutagen.

All of the biological end points described above make plants useful and even sophisticated mutagen detection and screening systems. Measuring frequencies of these end points can be made easier by creating mutant forms which facilitate detection of mutants, chromosome changes, etc. A mutant was recently uncovered in barley which exhibited better spread pachytene chromosomes than the normal strain—thus allowing easier analyses of chromosome abnormalities at this stage of meiosis (*79*). It is imperative that plant scientists working in mutagenesis be constantly improving the major plant test systems.

Tumors and Fasciations

In passing it should be mentioned that tumors and fasciations have long been observed in plants (*80, 81*). Many of these are of genetic origin while others are caused by a variety of insults to the plant. Morphologically, fasciations involve, in vascular plants, a change from a normal, round, or polygonal stem or axis, to one that becomes flat and/or ribbon-shaped. All parts of the plant have been recorded as altered through fasciation, but the most striking involves the axis of the plant. Typical fasciation or tumor formation has been recorded in at least 107 of the 303 families of plants. It occurs as commonly in wild as in cultivated plants, and in both hereditary and non-hereditary forms. It has been induced by x-rays, bacteria, and as indicated above, by numerous other causes in addition to mutations. As to the origin of fasciation there appears to be some concensus that they result from enlargement of a single growing point (*80*).

One of the best known bacteria induced tumors is crown gall. It is a neoplastic disease of many dicotyledenous plants initiated during infection by *Agrobacterium tumefaciens*. Transformation occurs within a few days after infection after which the presence of living bacteria is no longer necessary to maintain the tumorous state. Bacteria-free tumor cells can be isolated and cultured indefinitely on chemically defined media lacking the phytohormones normally necessary for growth of plant cells in culture.

Due to the excellent work of Eugene Nester's laboratory at the University of Washington, we now know that crown gall tumors are caused by the incorporation into the plant cells of a small part (3.7×10^6 daltons) of a virulent-plasmid carried by the inciting bacterium, *A. tumefaciens* (*82, 83*).

Fasciation or tumor formation bear an analogy to cancer from the standpoint that the same character is produced in a given organism by many different causes (*81*). It is probably of little value to attempt to draw further analogies at this stage. However, as yet no tumors have been induced by chemical mutagens and the whole problem of induction has received no attention. Because of their artificial as well as gene causation and their genetic modification, fasciations and tumors should be investigated further to determine if there is any aspect of this phenomenon that can be usefully analyzed to provide greater knowledge of the interrelation of mutagenesis and carcinogenesis.

Limitations of Plant Test Systems

At the outset it is important to realize that there are limitations to plants as systems for mutagen screening. Because of differences in metabolism, plants may have limited value for estimating risk/benefit of mutagens for man. Furthermore, as stated in the Introduction, the lack of knowledge at the molecular and ultrastructural level of so many plant processes that might affect mutagen action and mutant detection, put additional limits on the utility of plant systems in mutagenesis research.

The mode of access of a given mutagen to the target molecule in the expression of damage may be widely different in plant and mammalian systems. This would make the determination of actual quan-

tities of the mutagens in any comparative mutagenesis studies rather difficult.

Another problem of plant systems arises when one analyzes those chemicals which are first administered to the human body as pro-mutagens and are then transformed by enzymes into true mutagens. Dimethylnitrosoamine (DMN) and diethylnitrosoamine (DEN) can be cited as examples of this process as they are only effective in animals in the presence of liver homogenate. On the other hand, DMN is mutagenic in barley (*84*), *Arabidopsis* (*85*), and soybean (*86*) without addition of liver homogenate. It is also known that certain chemicals such as daunomycin and adriamycin are known to be ineffective in plant genomes but quite effective in mammalian cells (*87*).

The nature of mutations in most higher plant test systems is little understood. It is not yet possible to assign specific mutational events to specific mutants. Of course, all other major higher eukaryotic systems have this limitation as well.

There are no plant systems which have a life cycle as short as those of several major test systems such as *Drosophila*, yeast, and bacteria. *Arabidopsis*, with the shortest life cycle of any major higher plant used in mutagenesis, can yield mutation data one month after seed treatment. Even plant cell cultures can not yield reliable mutation data inside of six months. Somatic mutations can be scored in a plant such as soybean two weeks after mutagen treatment of the seed. Mutations induced at the *waxy* locus can be scored in pollen about one week after post meiotic mutagen treatment.

Role of Plant Test Systems in Mutagenesis Research

It is appropriate now to review briefly the role of plant test systems in various areas of mutagenesis research. Emphasis will be on the potential of plants as mutagen monitoring systems, but the roles plants have played in other aspects of mutagenesis research must not be ignored. These include detection and verification of mutagenic activity (mutagen screening); quantification of mutagenic activity; mechanism of mutagen action and nature of induced mutations; and risk/benefit evaluations of mutagens.

About 233 plants have been used in various aspects of mutagenesis research (*88*). Of these, the following have been widely employed: barley, broad bean, *Tradescantia*, *Arabidopsis*, corn, rice, tobacco, maize, onion, pea, soybean, tomato, and *Crepis capillaris*. Major genetic end points of some of these are presented in Table 2.

Detection and Verification of Mutagenic Activity (Mutagen Screening)

The mutagenicity of x-rays was first demonstrated in *Drosophila* by Muller in 1927, and within a few months in barley and maize by Stadler. Among the chemical mutagens, several were detected first in plants (*12*, *89*). More recently has been the discovery in our laboratory of the potent mutagenic activity of sodium azide in barley (*90*). This has led to demonstrations of its mutagenicity in bacteria, peas, soybean, *Tradescantia*, maize, mammalian cells, rice, and yeast in our and other laboratories. Its numerous uses in agriculture, industry, biological and chemical research, medical laboratories and hospitals, and more recently as a gas generator for safety airbags in automobiles makes sodium azide a major environmental mutagen.

Quantification of Mutagenic Activity

Unfortunately, the quantification of mutagenic activity received little attention in most plant systems. As indicated in the Introduction, the genetic effects of most mutagens have not been analyzed over a range of doses or concentrations, for least effective doses, and on a mutation rate per locus basis. With appropriate guidance and sufficient funds, this important mutagenesis activity should be increased in plants, especially with the more sensitive systems.

Mechanism of Mutagen Action and Nature of Induced Mutations

In the area of understanding mechanisms of mutagen action and the nature of induced mutations, the role of plant systems has been considerable in the former, but rather negligible in the latter.

In the early days of radiation genetics, Sax and his students in the U.S.A. and Catcheside, Darlington, La Cour, Koller, Thoday, and others in England, using such plants as *Tradescantia* and *Vicia*, provided new knowledge about the mechanism of chromosome breakage by UV, x-rays, and γ-rays (*58*, *91*). These and other plant systems have been used extensively to understand the mechanism of chromosome breakage by chemical mutagens (*12*, *13*, *91*).

Test systems, utilizing both mutation as well as chromosome aberration data, have been valuable in determining the influence of a variety of factors on the response of cells to physical and chemical mutagens. Some of the first investigations into the role of

factors such as metabolism, water and oxygen content, and temperature in the response of cells to sparsely and densely ionizing radiation were conducted with *Vicia faba* (*58*) and barley (*92, 93*). More recently, a number of important biological and physical parameters relating to the action of chemical mutagens, particularly the alkylating agents, in cells have been uncovered in barley (*2, 92, 94*) and *Arabidopsis* (*2, 95–99*).

Another dimension has been added to the value of plant systems for understanding mechanisms of mutagen action with the recent discovery that plants, like a number of other eukaryotic systems, form intermediary mutagenic metabolites following treatment with certain mutagens. The discovery of a mutagenic metabolite occurring in maize after atrazine treatment (*100, 101*) is now well known. Just recently, a mutagenic metabolite has been detected in barley embryos following sodium azide treatment in our laboratory (*102*). We also have some evidence that a metabolite of azide is responsible for its mutagenic action in Chinese hamster cells and bacteria. Enhanced mutagenicity of 1,2-dibromoethane (EDB) by plant metabolic activation has just been reported (*103*). DMN, which requires artificial activation in *Drosophila* and *in vitro* mammalian systems, acts without the liver homogenate in barley (*84*), soybeans (*86*), and *Arabidopsis* (*85, 99*). Useful papers for future studies of plant metabolic activation have been published (*104, 105*).

In terms of unravelling the nature of induced mutations, plant test systems have played very minor roles. Because of their low genetic resolving power, it has not been possible to determine the nature of induced mutations for any induced mutant. However, studies of *Adh* (*44, 45*) and *waxy* (*39*) loci in maize pollen have pointed the way toward more progress in this area.

Risk/Benefit Evaluation of Mutagens

Plant systems have played a very limited role in risk/benefit evaluations of mutagens to man. However, as plant mutation experiments become more sophisticated, as better test systems are developed, and as more quantitative data are obtained, plants will play a more significant role in this area of mutagenesis.

Mutagen Monitoring

Monitoring of mutagens in the human environment is an increasingly important activity in mutagenesis research. Mutagen monitoring with plants has two important objectives: first, to help determine potential hazards to man of air and water pollutants; second, to help protect the physiological and genetic purity of crop plants. This involves detecting changes induced by chemicals, e.g., pesticides, which can affect the food chain, and genetic changes, e.g., mutations for disease susceptibility, which can affect productivity.

The need is to detect, develop and utilize sensitive plant monitors for mutagen damage inside and outside of sources of pollution such as chemical manufacturing and research laboratories and *in situ* on land or in water. They must provide easily detectable genetic or chromosome changes. As indicated earlier, plants offer unique test systems for *in situ* monitoring, and yet progress in their use has been slow.

A certain amount of mutagen screening can be conducted in the laboratory, such as determining the mutagenesis of food additives, pesticides, etc., whether the chemicals be in a gaseous or liquid phase. It is also possible to set conditions in the laboratory that might simulate environmental pollution, whether it be in the atmosphere or in the water.

Plant Systems Already in Use as Mutagen Monitors. Two unique plant test systems are already in considerable use as mutagen monitors for air and water pollution. Mutations for color of the highly mutagen-sensitive *Tradescantia* stamen-hairs have been quite widely employed to monitor air pollution at a number of sites throughout the U. S. (*3*). Here a mobile laboratory is utilized through which ambient air is passed over *Tradescantia* cuttings and mutations are measured in a few days. Mutation and chromosome aberrations in an aquatic fern are indicating the degree of mutagenic pollution in Eastern rivers (*5–8*). Presently, no animal or other test systems are being utilized in the "real world" of mutagen monitoring.

Another form of mutagen monitoring is chromosome analyses of *in situ* weed species following pesticide treatments (*106*). The weedy species *Crepis capillaris* (Hawksbeard), with its three morphologically distinct pairs of chromosomes, should be ideal for *in situ* mutagen monitoring.

In the same vein, frequencies of *waxy* pollen mutants have been measured to determine the effects of a number of herbicides, including atrazine, on field grown maize plants (*100, 101*).

Developed Plant Systems that Should be Used in Mutagen Monitoring and Screening. Two well-developed plant test systems should be better utilized in monitoring mutagenic activities both inside and outside of air pollution sources. These are the small growing Crucifera, *Arabidopsis thaliana,* and cell cultures, particularly those of carrot.

The advantages of *Arabidopsis* for mutagen

screening and monitoring have been well described previously (*95–97*). These include the very small size of mature plants, self pollination, short life cycle (30 days), tremendous fecundity (50,000 seeds per plant), and rapidly scored mutational events in a relatively short period after mutagen exposure. This plant has been used extensively for mutagenesis research in relatively few laboratories. More important, its use as a mutagen monitor and extensive use for mutagen screening has been neglected. The mutagenic response of *Arabidopsis* to a number of chemicals has been reviewed (*107*).

Cultured plant cells seem to be a feasible system for the assay of genetic effects of pollutants occurring inside and even outside of sources (*108*). They appear to be more responsive to the gaseous phase of their incubation conditions. Furthermore, the temperature 20-30°C at which plant cells grow, is within the same range that the volatile compounds exert their effects in the natural environment. The plant cells' preference for low pHs (pH 4 to 6) is another advantage of plant tissue culture for mutagenesis studies. Many test systems have a functional range of pH 6 to 7, and yet compounds such as azide are known to be more mutagenic at low pHs. Since the human body exposes all ingested material to a strong acid environment in the stomach, suspected mutagens should be tested in acidic conditions. Haploid cell cultures have several obvious advantages for mutation and genetic experiments. The possibility of regenerating an adult plant from a single haploid cell is a major advantage of plant over animal cell cultures. Some of the recent investigations of mutation induction in haploid cells of *Crepis capillaris* and *Petunia* (*109*), and in polyhaploid cells of tobacco (*110*), have been summarized.

Single cell cultures of haploid carrots appear to have advantages over other cell cultures now in use for mutagen monitoring (*108, 111*). Haploid cells can be cultured into plants to test mutant types. Plants regenerated from haploid cells can be treated with colchicine to produce a diploid branch which will flower and produce seeds. This permits the presumptive somatic mutations to be analyzed via sexual reproduction. The growth of a vast number of cells on defined media facilitates mutant isolation via selection, providing an ideal system for quantitative assay. These cells are suitable for testing the mutagenicity of volatile compounds and plant mediated metabolites.

The production of auxotrophs leads to one measure of mutagenic activity. Once auxotrophic mutants are generated their reversion frequency to protrophs can be employed to test for mutagenicity of environmental chemicals.

Plant Systems That Should be Developed for Mutagen Monitoring and Screening. POLLEN GENES: The biology of the male gametophyte or pollen of Angiosperms has been thoroughly described (*112, 113*). As indicated previously, the male gametophyte provides numerous advantages as a test system for mutagen screening. These include scoring mutational events based on large cell populations, thus simulating microbial mutation and genetic studies. *Waxy* (*wx*), self-incompatibility (*S*), and alcohol dehydrogenase (*Adh*) are genes whose mutants have been utilized to measure mutation frequency following mutagen exposure. In addition, chromosome aberrations can be scored following microsporocyte or microspore treatment. Other details of genetics and mutagenesis studies of pollen traits have been described (*42, 44*).

The potential of the self-incompatibility (*40*) and the *Adh* (*44*) gene systems for mutagen monitoring were described at this Workshop. Improvements are underway in the *waxy* gene system of maize toward greater use in mutagen monitoring (*101*).

WAXY POLLEN TESTER IN BARLEY: The *waxy* gene in barley is another pollen system that should be utilized more extensively. It is a single locus at the distal end of the short arm of chromosome 1. It has all of the advantages of a pollen system plus the fact that meiosis in barley is quite synchronous. The latter permits treatment of microspores and scoring for *waxy* mutants and chromosome aberrations in the pollen at precise times. The barley plant is easily grown, highly adaptable, and occupies relatively little space and is thus adaptable to mobile monitoring units, research and industrial laboratories, and fields around sources of pollution. It has been employed as a monitor for ethylene oxide (*115*).

At Washington State University a mutagen monitoring and screening system utilizing mutations of the *waxy* pollen locus is being developed. This system will analyze revertants of mutants of known mutation origin, thus providing information on the nature of the mutations induced by airborne mutagenic agents in the laboratory or the field.

Twenty-five *waxy* mutants, most representing different alleles, have been induced by azide and γ radiation. These alleles will be treated with a set of mutagens such as EMS (base substitution), azide (base substitution), and proflavin azide (frame shift) to identify at least one allele that reverts with a base substitution and one that reverts with a frame shift mutagen. Additional alleles will be induced by specific mutagens. Revertants will be scored among millions of pollen grains by the iodine staining technique.

Alleles selected for detecting the nature of muta-

tions will be transferred to a short-strawed, early maturing line that will reduce the time for mutant scoring and allow more plants to be grown in a confined space. This test system would be unique among higher eukaryotes as it would not only be highly sensitive to mutagenic activity but also provide information on the nature of the mutations induced.

WHITE CLOVER (Trifolium repens): At present there is not an adequate *in situ* monitoring system among plants and animals by which mutagenic activity of the environmental components can be monitored year in and year out. A plant, preferrably a perennial, is required that can remain on site for a number of years and provide mutation data each year. One candidate is white clover.

White clover is a perennial and the use of this organism in mutagen monitoring is based on somatic mutations that can be induced at the leaf marking locus. It is a true diploid with a chromosome number of $2n = 32$. There are eleven alleles at this locus which result in the production of different phenotypes at the leaf marking locus (*116*). The allele most frequently used in mutagen studies (*117–120*) is V^{bv} which produces a broken white V surmounted by a yellow tip on leaves.

Heterozygous V^{bv} b lines can be easily cloned vegetatively to produce thousands of tester plants for field planting. Since it is highly prolific it fills fields very rapidly.

Mutations at the V^{bv} allele produce the homozygous condition which results in the complete absence of a leaf mark. The locus appears to have no physiological effect, thus mutants are not at a disadvantage.

It has advantages for mutation experiments in that large numbers of leaves can easily be produced and examined for mutants. Mutation frequency is determined in terms of occurrence of leaves exhibiting mutants. Radiation dose curves (*119*) indicate this system is quite sensitive. Work with other alleles at this locus may produce more mutagen sensitive strains.

Dose response curves and fractionated dose experiments suggest that a wide range of mutations from true gene or point mutations to partial or whole chromosome losses are probably involved in mutations at this locus. No spontaneous mutations were found among thousands of the control plants.

The time of mutant appearance after mutagen treatment of the meristems varies according to the condition of growth from four weeks in the summer to ten weeks in the winter.

Aquatic Monitors

Aquatic monitoring of mutagenic pollutants is another neglected area of mutagenesis research. At present the fern, as used by Klekowski (*5–8*) is the only well-developed sensitive system for aquatic monitoring. There are however, numerous aquatic plants some of which might be suitable, with some genetic manipulations, for this activity.

One candidate might be within the *Lemnaceae*. This family comprises a number of species of small floating plants commonly called duckweed (*121*). The species of several genera grow in temperate and tropical zones in fresh or somewhat saline, often highly polluted water. They are the simplest and smallest of flowering plants consisting of a leaf and a root.

Individual plant bodies, termed "fronds," are barely more than 3 mm thick, and range from 1 mm to 1.5 cm in length or diameter. The flowers are reduced to a single pistil, and one or two stamens. All species have vigorous vegetative reproduction, and in the common buckweed (species) flowering is quite frequent. In other species, flowering is infrequent. The rapid vegetative growth can provide the biochemist with essentially unlimited supplies of material growth under specified conditions. The small bulk and floating habits of duckweeds mean that compounds in the medium are at most a few cell layers away from any part of the plant. Thus, these plants have been excellent experimental materials for physiological studies (*122, 123*) and could possibly be developed for mutagen monitoring as well.

They are diploid (ca. $2n = 40$) and the chromosomes are extremely small. Breeding experiments that might be conducted to make these plants more suitable for both physiological and genetic studies have been suggested (*124*). Mutant auxotrophs have been apparently induced by x-rays. These mutants may be due to dominant mutations or chromosomal aberrations (*125*).

The water hyacinth (*Eichhornia crassipes*) is a sensitive biological indictor for continuously monitoring trace quantities of toxic heavy metals in aquatic systems (*126*), and is becoming increasingly valuable as a water purification system in cities in the U. S. It is self-fertile and a tetraploid. Its possible use as a mutagen monitor should be explored.

Summary and Conclusions

Plants have too long been ignored as useful screening and monitoring systems of environmental

mutagens. In truth, there are about a dozen reliable, some even unique, plant systems that can increase the scope and effectiveness of mutagen screening and monitoring procedures. Plants are the only systems now in use as monitors of genetic effects caused by polluted atmosphere and water.

The major advantages of plants that should be considered in mutagen screening and monitoring are (1) chromosome structure similar to mammals, including man, (2) responses to given mutagens similar to those of other non-mammalian and mammalian systems, (3) regeneration from single haploid and diploid cells, (4) mutagen sensitive with short life cycles, (5) relatively inexpensive to culture and obtain data, and (6) *in situ* monitoring.

In addition to these advantages, the major plant test systems have numerous genetic end points for determining the effects of mutagens. Some of these have not yet been detected in other nonmammalian and mammalian test systems, but probably occur in the human organism. The mutational events include mutations at multiple and specific loci, cytoplasmic mutations in chloroplast and mitochondria DNA, controlling elements and paramutations, single-strand DNA breaks, and DNA repair. The chromosome changes include chromosome and chromatid breaks, aided by low numbers of morphologically distinct chromosomes, reconstructed karyotypes and banding techniques; subchromatid aberrations; somatic crossing over; aneuploidy from nondisjunction; sister chromatid exchanges, micronuclei; and chiasmata. Certain of the plant systems permit several of these genetic end points to be scored following a single mutagen treatment, thus providing a more realistic and in-depth interpretation of the effects and action of a given mutagen. Highly sensitive test systems are available when using genes which control pollen structure and chemistry.

Plants have played major roles in various aspects of mutagenesis research, primarily in mutagen screening (detection and verification of mutagenic activity), mutagen monitoring, and determining mutagen effects and mechanisms of mutagen action. They have played lesser roles in quantification of mutagenic activity and understanding the nature of induced mutations.

Mutagen monitoring with plants, especially *in situ*, on land or in water, will help determine potential genetic hazards of air and water pollutants and protect the genetic purity of crop plants and the purity of the food supply. The *Tradescantia* stamen-hair system is used in a mobile lab for determining the genetic effects of industrial and automobile pollution in a number of sites in the U. S. The fern is employed for monitoring water pollution in the Eastern states. It is proposed that (1) the small Crucifer *Arabidopsis* be more widely utilized, (2) weedy species such as *Crepis capillaris* with morphologically distinct chromosomes be developed as *in situ* chromosome-breakage monitors, (3) haploid cell cultures of the carrot be developed for monitoring pollution sources inside laboratories and factories, (4) pollen genes be utilized more effectively in plants such as barley and maize for developing monitoring and screening systems which cannot only record low levels of genetic effects but can also provide information on the nature of mutations induced by environmental mutagens, (5) white clover, a perennial which has very distinct mutation markers, be utilized for long-term *in situ* monitoring of atmospheric pollutants, and (6) plants such as duckweed and water hyacinth be developed as monitors of mutagenic pollutants in rivers and lakes.

It is concluded that plants have already demonstrated their usefulness in mutagenesis research, including mutagen screening and monitoring. However, their potential in this area is not yet realized nor is it generally recognized. This potential will be realized in part by improving knowledge of plant cell processes at the molecular and ultrastructural levels, relating these processes to mutagen effects and plant cell responses, improving current plant test systems for increased sensitivity, ease of detecting genetic end points, recording of data (including automation), and extending the range of genetic end points, and designing and developing new systems with the aid of previous and current botanical and genetic knowledge.

Well-conceived mutagenesis experiments yielding quantitative data that permit valid comparisons of responses to given mutagens with other test systems, greater recognition of the value of plant test systems by more scientists and granting agencies, and more funds for basic plant research will also lead to placing plants in their rightful role among tier test systems and as valuable monitors of the genetic hazards of environmental mutagens. Finally, it is maintained that this workshop has brought together for the first time appropriate scientists engaged in the development of improved mutagen monitoring and screening plant systems. This type of interaction must be fostered for further development of efficient and effective plant systems for all aspects of mutagenesis research.

Some of the information reported was obtained from research conducted under Department of Energy Contract No. EY-76-S-06-2221.

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