Utility of Specific Locus Systems in Higher Plants to Monitor for Mutagens
by Milton J. Constantin*

Plants possess biological and operational attributes that have encouraged geneticists to use them extensively in the development of fundamental genetic concepts. Attributes such as regenerative plasticity, high fecundity, cultural adaptability, range of ploidy, economics of culture and maintenance of specific populations, and versatility make plant genetic systems prime candidates with which to monitor the environment for mutagens.

A specific locus (equivalent to a classical Mendelian gene) controls the development of a phenotypic characteristic. It can also mutate to a new allelic form with a consequentially altered phenotypic characteristic and can be separated by crossing over from adjacent loci that govern other specific phenotypic characteristics. Since various plant species have numerous specific loci, one has a rich array of potential systems from which to select.

Specific locus systems in higher plants could be used to assess the mutagenicity of single chemical compounds or combinations of chemical compounds. Depending on the circumstances, seeds and/or seedlings could be used; plants could be grown in situ in either containers or plots to assess the immediate environment for one or more mutagens over an extended period. Since plants are eucaryotes, data from such experiments could serve as one more source of information along with that obtained from a battery of other tests used in the tier system.

Introduction

Specific locus systems in higher plants have been used for experiments with chemical and physical mutagens. Generally, the objectives have been to assess methods of treatment and cultural management relative to their application in mutation breeding for crop improvement. However, the purpose of this Workshop is to evaluate the potential of higher plant systems to monitor the environment for mutagens. I should therefore like to emphasize attributes of higher plants as potential systems to monitor for mutagens, describe three specific locus systems which are not covered otherwise in this Workshop program, and define the role that specific locus systems in higher plants can play in assessing the mutagenicity of pesticides, industrial pollutants, etc.

Plant Genetics Relative to Other Fields of Genetics

In Genetics of Flowering Plants, Grant (1) devotes one chapter to a comparison of the impact of plant genetics with that of molecular, microbial, population, and human genetics in relation to their actual and potential role in modern biology. Grant emphasizes that plant genetics no longer enjoys the dominant position in the overall science of genetics that it once did. In addition to being the organism chosen for Mendel’s pioneering experiments which formed the foundations for the science of genetics, plant systems have been used in numerous experiments, the results of which have contributed significantly to the development of fundamental concepts. I would like to view these past accomplishments in the context of what can be gained in terms of understanding the role that plants can play in monitoring environmental quality.

Attributes of Higher Plants as Monitors of Mutagens

Let us consider some attributes of higher plants that have induced geneticists to use them extensively in the past. Which of these attributes are of significant value today in monitoring the environment for mutagens?
Biological Attributes

Regenerative Plasticity. The numerous modes of reproduction in plants, including sexual and asexual propagation (frequently within the same individual), permit unique populations to be established. For example, controlled crosses can be made readily to obtain desirable heterozygous genotypes that can then be reproduced vegetatively to establish clones for genetic studies. Rick (2) discusses this topic in relation to the tomato as a species for genetic studies.

Fecundity. Large numbers of seeds from which offspring can be derived, generally at will, are produced by a single plant and in some cases by a single ovary. For example, a single plant of Arabidopsis can yield over 50,000 seeds (3), one of Mimulus cardinalis over 200,000 seeds (4), and one of tobacco over 1,000,000 seeds (5).

Cultural Adaptability. Many plant species can be cultured successfully under a wide range of environmental conditions from axenic culture in laboratory test tubes to pot culture either indoors or outdoors to extensive plantings in the field.

Generation Time. The time required to complete a life cycle varies from one month for Arabidopsis (3) to many years for certain trees. Certain genetic systems do not require a complete life cycle for use in experiments.

Ploidy. A wide range of ploidy levels exists in nature among species of plants; however, ploidy can be manipulated readily within a species. High polyploids are frequently fertile, and haploids can be reproduced asexually. In vitro culture techniques offer the potential for developing haploids in sufficient number for experimental purposes (6). Certain plants including Allium, Lilium, Tradescantia, Vicia, and Hordeum have both large chromosomes and few chromosomes which facilitate cytogenetic studies.

Developmental Morphology. The relatively simple ontogeny found in plants permits various chimeras that are not found in other kinds of organisms. Some of these chimeras can be used advantageously in genetic studies (7).

Operational Attributes

Economics. Culture of large populations of plants under either field or laboratory conditions can be achieved with relative ease and little expense compared to culture of animal populations of similar size. Facilities required for plant populations are less extensive than those for animal colonies (7).

Maintenance of Organisms for Experiments. Seeds, vegetative reproductive structures, and now in vitro cultured cells can be kept in long-term storage to circumvent significant investments to maintain test organisms. Storage of large inventories is feasible, making available a ready supply of uniform population for test purposes over a long period.

Versatility. Seeds and certain vegetative reproductive parts can be handled with little regard for time and temperature. One can utilize seeds, for example, to study the interaction of physical and chemical agents as expressed at the genetic level (7). Factors such as anoxic atmosphere can be tolerated with relative ease by seeds, whereas the same conditions would be either toxic or lethal to most animals. Populations of plants can be grown at sites far removed from laboratory facilities for relatively long periods of minimum attention to site preparation, maintenance, etc.

Applicability of Experimental Data

The present tiered system approach for testing the mutagenicity of various compounds attests to the problems inherent in extrapolation of data from one system to another. It must be emphasized, however, that the degree of genetic complexity in higher plants is comparable to that which exists in humans. Indeed, both humans and higher plants are eucaryotic organisms, so their chromosome structure and biochemistry may be similar (8). Other similarities include DNA content per cell, extent of repetitive DNA, regulation of gene action relative to ontogeny, numbers of genes, etc. From a historical perspective, those fundamental genetic concepts based on results of experiments with plants have been found for the most part to be applicable to animals. Of course, one cannot ignore the problems associated with species differences caused by metabolism, routes of ingestion, metabolic activation, etc.

Definition of Specific Locus

A specific locus is equivalent to a gene in the classical Mendelian sense. As such, a specific locus is defined as a chromosomal region that controls the development of a phenotypic characteristic, that can mutate to a new allele with a consequentially altered phenotypic characteristic, and that is separable by crossing over from adjacent loci governing other specific phenotypic characteristics.

Specific Locus Systems in Higher Plants

Numerous specific loci, according to the definition given above, have been characterized in plant
species. In fact, reference is made to 250 loci associated with the seven linkage groups of barley (7), over 1100 genes in the cultivated tomato (2), over 300 loci for maize (9), 55 genes in Cucumis (10), and 500 loci readily available in peas (11). Obviously the scope of this report does not permit complete coverage of the literature dealing with these systems.

Plants such as Antirrhinum, Oenothera, Zea, Arabidopsis, Tradescantia, Lycopersicon, and Hordeum have been used extensively for mutagenesis experiments involving both chemical and physical mutagens. Frequently the protocol for these experiments is similar, if not identical, to that which would be required to monitor for mutants.

The Chlorophyll Deficiency System in Barley

The Organism. Hordeum vulgare L. emend. Lam., cultivated barley, is a diploid with 2N = 14 chromosomes that are relatively large and easily identified. The plant is self-fertilized; thus recessive mutations are not lost through outcrossing. Hybridization is accomplished easily which makes genetic studies relatively easy to complete.

The System. Cultivated barley was used quite early in studies of mutation induction by ionizing radiation. Discrete phenotypes involving either a deficiency or lack of chlorophyll were observed frequently in segregating generations following treatment with mutagens. These phenotypes were described and classified by Gustafsson (12) in 1940. Numerous gene loci are responsible for these chlorophyll-deficient phenotypes; estimates include 250–300 (13), and several hundred (14) for the Albina phenotype alone. Most of these chlorophyll-deficient mutants behave as monofactorial recessives. Constantin (15) recently reviewed this system.

Procedures. The barley system has been used for various types of experiments involving mutagenesis. One involves the treatment of seed with either a single mutagen or a combination of mutagens. When seeds are treated, the M1 generation plants can be used to assess morphophysiological effects such as degree of reduction in seedling height, survival to maturity, fertility, etc. The M2 generation is used to assess the degree of genetic effects usually based on the frequency of chlorophyll-deficient phenotypes. This approach measures genetic effects in germinal tissue. It is relatively simple to relate genetic effects induced by a mutagen (pollutant) observed in the M2 generation to the toxic effects induced by the same mutagen observed in the M1 generation.

The dormant embryo of barley has up to four leaf primordia and two visibly differentiated axillary buds in addition to the shoot apex. Each plant can develop up to six primary tillers from distinct primordia plus numerous higher order tillers. A mutation induced in a cell of one primary tiller will not be expressed in any other primary tiller. Thus chimeras are to be expected at the M1 plant level. In addition, from one to four initial cells participate to form a spike; thus chimeras are to be expected within a spike. In fact, chimeras have been observed at the individual anther level. It should be noted that cells of the dormant embryo (dry seeds) are arrested predominantly in G1 phase of the DNA cycle; however, when soaked seeds are treated the duration of the soaking period will determine the phase of the DNA cycle for the majority of cells. This influences the degree of genetic effects.

Another type of experiment involves the exposure of plants to a mutagen throughout an entire life cycle. Ionizing radiation has frequently been the mutagen of choice in such experiments. In this case, the M1 plants (the generation of individuals grown in the presence of the mutagen) are grown to maturity and harvested individually. M1 progeny is grown from each M1 plant in the absence of the mutagen, and individual M2 plants are harvested. The M2 generation is used to determine the frequency of chlorophyll-deficient phenotypes. In this type of experiment, one measures an effect integrated across different developmental stages which might vary considerably in their vulnerability to the mutagen.

Results. Figure 1 shows chlorophyll-deficient mutants per 106 M2 seedlings of Himalaya barley induced by various concentrations of ethylmethane sulfonate and the computed linear regression; data were taken from the report of an experiment by Konzak et al. (16) and reproduced from (15). Similar data for Atlas-57 barley following the treatment of seeds with either gamma radiation or unmoderated fission neutrons are shown in Figures 2 and 3; data were taken from the literature (15, 17–19). These data show conclusively that the barley system will yield mutants following seed treatment with either chemical or physical mutagens and that the response is linear for the range of treatments used in these experiments.

The barley system has also been used in experiments involving exposure of plants throughout their life cycle to various levels of γ-radiation. One such experiment was conducted using Atlas-57 plants grown in the gamma garden at Brookhaven National Laboratory (20). The M0 plants were exposed to γ-radiation for 20 hr/day from the time of emergence to the time of harvesting (a total of 96 days). Exposure rates ranged from 0.17 R/day (16.5 R total ex-
posure) to 23.2 R/day (2240 R total exposure). Five spikes were harvested from each M<sub>1</sub> survivor (2200–3000 M<sub>1</sub> seeds planted per treatment) and planted in greenhouse beds for scoring for chlorophyll-deficient phenotypes. Data are shown in Table 1. Number of mutants per 10<sup>4</sup> seedlings increased in an approximately linear fashion as the exposure per day increased from 0.17 to 23.2 R/day. Even the lowest exposure per day increased the mutant frequency from 1.3 to 4.3 per 10<sup>4</sup> seedlings.

Table 1. Mean number of chlorophyll-deficient phenotypes per 10<sup>4</sup> M<sub>2</sub> seedlings relative to γ-radiation exposure during the life cycle of the plant.<sup>a</sup>

| Exposure rate, R/day | Exposure, R | Mutants/10<sup>4</sup> M<sub>2</sub> seedlings |
|----------------------|-------------|----------------------------------|
| 0                    | 0           | 1.3                              |
| 0.17                 | 16.5        | 4.3                              |
| 0.34                 | 32.0        | 3.9                              |
| 0.73                 | 69.5        | 4.5                              |
| 1.45                 | 139.2       | 4.1                              |
| 3.17                 | 303.7       | 7.0                              |
| 7.15                 | 692.3       | 24.9                             |
| 12.76                | 1235.0      | 23.3                             |
| 23.20                | 2240.0      | 45.4                             |

<sup>a</sup> Data of Conger et al. (20).

The Y<sub>G</sub> System in Maize

The Organism. Maize (Zea mays) is a diploid with 2N = 10 chromosomes. The plant is a monoecious summer annual that is mostly cross-fertilized by wind-dispersed pollen. Because the plant has separate pistillate and staminate inflorescences, both selfing and hybridizing can be achieved readily through the use of appropriate bags. Maize has been used extensively in genetic studies; thus numerous loci have been identified and assigned to specific chromosomes. For more details refer to Neuffer and Coe (9).

The System. The Y<sub>G</sub> locus is found near the end of chromosome 9. Leaf color is normal (green) in the homozygous dominant and in the heterozygote but is abnormal (yellowish green) in the homozygous recessive. The loss (deletion) or alteration of function (mutation) of the dominant allele in
the heterozygote results in the yellowish green phenotype. When seeds or young seedlings are exposed to mutagenic agents that induce either a deletion or mutation of the dominant allele, yellowish green sectors appear on the leaves. The younger the leaf primordium is at the time of treatment, the larger the sectors are; conversely, the older the leaf primordium is, the smaller the sectors are. Older leaves have more sectors than do younger leaves. The mature maize embryo in the dormant kernel possesses six leaf primordia. For more details refer to Stein and Steffensen (21) and Smith et al. (22).

**Procedures.** Generally, seeds heterozygous for the Yg8 locus are treated with a mutagenic agent, and plants are grown for approximately one month. The frequency of yellowish green sectors is then determined for leaves 3, 4, 5, or 6. Growing the plants at a relatively cool (60-75°F) temperature helps in the identification of sectors.

**Results.** The first results are from an experiment by Smith et al. (23) in which dry seeds (6.7% water content) were exposed to 250 kVp X-rays. The X-ray doses, the number of leaves scored, and the mean frequency of sectors per leaf 4 are shown in Table 2. The relationship between X-ray dose and mean frequency of sectors on leaf 4 was linear. No relationship was detected between dose rate (455 to 980 rad/min) and the frequency of Yg8 sectors. Likewise, no post-irradiation storage (up to 14 days) effect was detected.

The second results are from an experiment by Conger (24) in which soaked seeds were irradiated with either 60Co γ-rays or unmoderated fission neutrons. Dose of γ-rays and fission neutrons, number of leaves scored, and mean number of sectors per leaf 4 are shown in Table 3. Soaking the seeds for 96 hr prior to irradiation allowed the expansion of existing leaf primordia, but no new organs were initiated. Cytophotometric studies revealed that 33.2, 16.4, and 39.5% of the cell population scored (shoot apex and leaves 4, 5, and 6) were in G1, S, and G2, respectively. In comparison, dry seeds had a greater fraction of the cell population in G1 (24). In the case of the presoaked seeds, a log-log relationship existed between radiation dose and the mean frequency of sectors per leaf 4.

In this system the Yg8 sectors are generally assumed to be the result of a chromosomal deletion involving the short arm of chromosome 9. This assumption is further supported by the results when ethyl methane sulfonate is used compared to those when sodium azide is used to treat seeds (25). Ethyl methane sulfonate can cause chromosomal aberrations, whereas sodium azide does not. In the Yg8 system, ethyl methane sulfonate induces a high frequency of sectors, whereas sodium azide induces a low frequency.

**The Thiamine Auxotrophy System in Arabidopsis**

**The organism.** *Arabidopsis thaliana*, a short life cycle (= 30 days) member of the Cruciferae family, is a self-fertilized diploid with 2N = 10 chromosomes. *Arabidopsis* has been used as the test organism for numerous experiments involving mutagenesis. The plant offers several advantages including a number of marker loci, a complete life cycle requiring from 30 to 50 days, a small size permitting large numbers of plants to be grown in a

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**Table 2. Mean frequency of Yg8 sectors per leaf 4 of heterozygous maize plants induced by X-irradiation of dormant seeds.**

| Dose, rad | Dose rate, rad/min | Mean number of sectors/leaf 4 |
|----------|-------------------|------------------------------|
| 1,500    | 455               | 0.20                         |
| 1,590    | 980               | 0.40                         |
| 2,000    | 980               | 0.32                         |
| 3,000    | 455               | 1.09                         |
| 3,000    | 980               | 1.89                         |
| 4,000    | 980               | 1.82                         |
| 4,500    | 455               | 1.29                         |
| 6,000    | 980               | 2.29                         |
| 6,000    | 455               | 4.03                         |
| 6,000    | 593               | 5.01                         |
| 7,500    | 455               | 3.29                         |
| 7,650    | 980               | 5.43                         |
| 9,000    | 455               | 4.20                         |
| 9,000    | 593               | 7.02                         |
| 15,600   | 980               | 11.89                        |

* Data of Smith et al. (23).

**Table 3. Mean number of Yg8 sectors per leaf 4 of heterozygous maize plants as affected by dose of γ radiation and fission neutrons to presoaked seeds.**

| Exposure       | Dose, rad | Sectors per leaf (mean ± S.E.) |
|----------------|-----------|-------------------------------|
| None           | 0         | 0.00 ± 0.00                   |
| Fission neutrons | 0.5     | 0.13 ± 0.06                   |
|                | 1.0       | 0.23 ± 0.08                   |
|                | 2.0       | 0.68 ± 0.16                   |
|                | 4.0       | 0.78 ± 0.12                   |
|                | 7.0       | 0.97 ± 0.23                   |
|                | 16.0      | 2.03 ± 0.36                   |
|                | 23.0      | 3.56 ± 0.39                   |
|                | 32.0      | 5.25 ± 0.50                   |
| γ-Radiation    | 30        | 0.13 ± 0.04                   |
|                | 60        | 0.39 ± 0.12                   |
|                | 120       | 0.83 ± 0.12                   |
|                | 240       | 2.60 ± 0.33                   |
|                | 360       | 4.57 ± 0.47                   |
|                | 480       | 6.90 ± 0.58                   |
|                | 600       | 9.63 ± 0.53                   |

* Data of Conger (24).
Role of Specific Locus Systems in Higher Plants to Monitor the Environment of Mutagens

The present concept of testing for mutagenicity involves a tiered-system approach including both prokaryotic and eucaryotic organisms. These organisms are used as surrogates for humans, and any substance which yields positive results is suspect. Plant systems such as Arabidopsis, Zea, Hordeum, etc. can serve as another data point in the assessment of mutagenicity. Plant systems offer several advantages including the ability to test the environment in situ for the presence of mutagens. The climatic conditions that prevail in the area to be monitored will dictate to some extent the kind of plant that can be used without the benefit of environment-controlled chambers. Plant systems are available that vary from a single specific locus to numerous specific loci that can monitor a site over several days in the case of Arabidopsis to several weeks in the case of Hordeum.

Plant systems are also available that can be used to assess the mutagenicity of chemical compounds either alone or in combinations in laboratory-type experiments. In addition, some plant systems can be used advantageously to monitor for chromosomal aberrations and toxicity.

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