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Somatic Mosaicism in Plants with Special Reference to Somatic Crossing Over

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Plant systems in use for the detection of environmental mutagens appear capable of detecting all types of genetic effects which can be studied in animals. The study of somatic mosaicism, however, is better developed in plants than in higher animals. A case is presented here which shows the ability of plant systems in analyzing a host of genetic end points, including chromosome aberrations like deletions, somatic crossing over, numerical inequality, gene conversion, paramutations and point mutations. The systems in general use utilize certain varieties of *Tradescantia, Glycine max, Nicotiana tabacum, Antirrhinum majus, Petunia hybrida,* and *Arabidopsis thaliana.* Heterozygous plants or their homozygous counterparts with gene markers affecting chlorophyll development or anthocyanin in floral parts are exploited in these studies.

Mutagens produce different frequencies of different types of spots typical of the mode of action of the agent. Analysis of these parameters may be used to predict, at least qualitatively, the kind of genetic damage that might be produced in man. Besides, one can test the validity of interpretation by traditional progeny tests of plants raised from tissue culture from sectors as in *Nicotiana* and/or by precursor analysis as done in *Antirrhinum.* The study of mosaicism in plants offers quite inexpensive, rapid, and reliable tests of mutagenicity at least as a preliminary eukaryotic test system.

Introduction

A variety of plant and animal species express mosaicism which is either a natural phenomenon under the normal control of the genetic system of the organism or is induced as an aberration resulting from the influence of environmental factors. We are interested in the latter as a tool in the study of environmental mutagenesis.

Mosaicism, mostly as spots on leaves or petals or as variant cells in stamen hairs, has been exploited in such studies. A great advantage is that the sectors showing alterations are being studied against the "native", *in situ* background. This situation minimizes the effect of variations due to cultural conditions. Also, one is scanning thousands of cells when one looks at a spot and its surrounding tissue. Another great advantage is that the affected cells do not enter a stringent competition as is visualized for other tissues of the plant. Most of the colonies of cells expressing as spots are only a few generations removed from the cell which was effected. It is only the question of a few cell divisions and expansion. Somatic sectors produced by a mutagen are good indicators of mutagenic ability of the agent on meiotic cells (*1*). These systems are very rapid, inexpensive, and quite reliable, at least, in terms of qualitative aspects of the information one can obtain about mutagens.

Unfortunately, and partially the result of neglect of plant systems in such studies, one has only a limited number of species to cite examples from. Most of the work has been carried out with *Tradescantia hirsuticaulis, Glycine max, Antirrhinum majus, Pisum sativum, Nicotiana tabacum, Zea mays,* and *Arabidopsis thaliana.* A list of promising plant species is reproduced in Table 1.

The Soybean System

I shall now take a few specific examples on the nature of mosaicism and quantitative results obtained. Because of my familiarity with the soybean (*Glycine max*) system, I shall first discuss it in somewhat detailed manner. The occurrence of

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Table 1. Higher plant systems promising for the study of mutagen-induced mosaicism, including twin spots.

Species	Tissue	Genes involved	Spontaneous spot frequency	Reference
Antirrhinum majus	petal	*niv, inc, pal, eos*		Harris and Carpenter (*2*)
Arabidopsis thaliana	leaf	ch^1 *(pa)*		Ahnstrom et al. (*3*)
				Hirono and Redei (*4*)
Collinsia heterophylla	leaf	w_1		Zaman and Rai (*5*)
Glycine max	leaf	y_{11}	0.08/leaf	Evans and Paddock (*6*)
Gossypium barbadense	leaf	v_1, v_7	0.03/leaf	Barrow et al. (*7*)
Hordeum vulgare	leaf			Nilan (unpublished)
Lycopersicon esculentum	leaf	xa_2	0.01/leaf	Ross and Holm (*8*)
Nicotiana tabacum	leaf	a_1, a_2, *t, su*	0.15/leaf (for *su*)	Deshayes and Dulieu (*9*)
				Carlson (*10*)
Petunia hybrida	leaf, petal	*a, b, p, r*	vo	Cornu (*11, 12*)
Salvia splendense	petal	*v, l, int, p*	0.3/petal	Hendrychova-Tomkova (*13*)
Tradescantia hirsaticaulis	stamen hair, petal	*d, e*	0.001/stamen hair	Cuany et al. (*14*)
				Sparrow et al. (*15*)
				Mericle and Mericle (*16–18*), Christianson (*19*)
Zea mays	endosperm, leaf	*c, pr, su, wx, yg*	0.039/kernal	Nauman (*20*)
				Jones (*21*)
				Conger and Carabia (*22*)

somatic crossing over and other chromosomal processes leads to mosaicism on leaves (*23–26*). The gene combination of $Y_{11}y_{11}$ has been used as a marker system. In varieties L65-1237 and T 219, the $Y_{11}Y_{11}$ plants are dark green in color, $Y_{11}y_{11}$ are light green, and $y_{11}y_{11}$ are golden yellow. The two simple leaves and, occasionally, the first compound leaf of the $Y_{11}y_{11}$ plants may exhibit some dark green (resembling the phenotype of $Y_{11}y_{11}$ leaves), yellow (like $y_{11}y_{11}$ leaves), or twin (double) spots (Fig. 1). (The latter are composed of a dark green component placed adjacent to, and almost mirror image of, a yellow component.) These double spots are inferred to originate by somatic crossing over leading to $Y_{11}Y_{11}$–$y_{11}y_{11}$ type of sectoring on the $Y_{11}y_{11}$ background. Some of the single (dark green or yellow) spots may have their origin in the failure of one component of twin spots; others may be due to chromosomal segmental losses or numerical inequalities in chromosome distribution during mitosis (*27*), or simple point mutations (*23, 28*). Thus, an increase in the frequency of double or simple spots on these heterozygous leaves may reflect the capability of an agent for inducing somatic crossing over or gain/loss of a partial or complete chromosome. An increase in the frequency of light green spots on the $y_{11}y_{11}$ leaves (Fig. 2) will point to the induction of mutation at the y_{11} locus. The system, therefore, has the advantage of testing new mutagens and, also, of detecting the various modes of action of these mutagens, all by one treatment.

In order to define the "discriminatory sense" of the system I shall consider the chemical mutagens and radiations tried according to the spectrum of changes these produce. The case of NaN_3 may be a

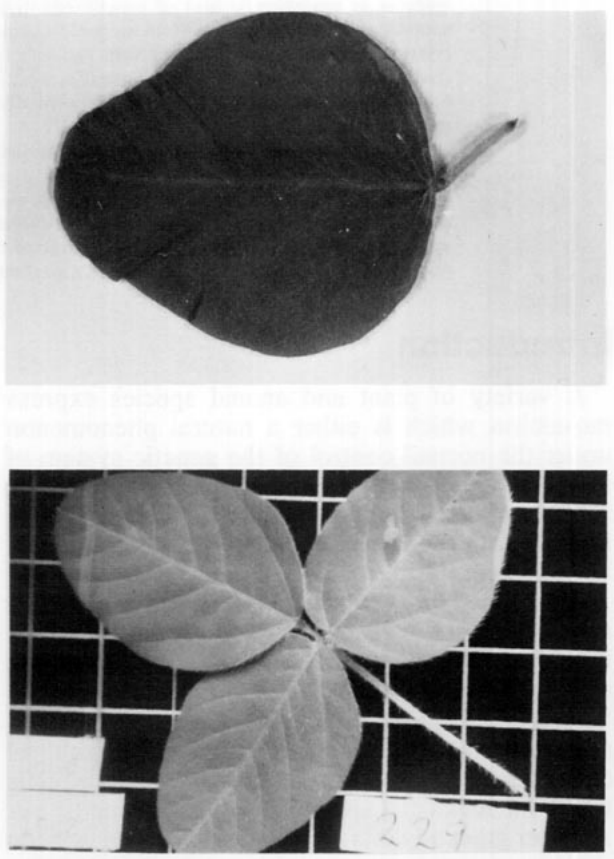

FIGURE 1. (*a*) a simple leaf from the variety T219 of *Glycine max* showing a small dark green, a large yellow and a medium sized twin spot; (*b*) a twin spot on the first compound leaf from seed treated with mitomycin C.

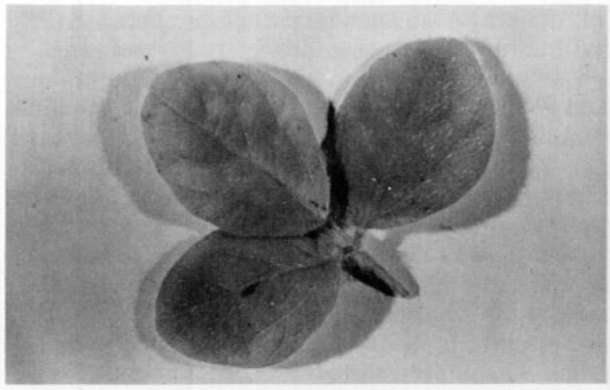

FIGURE 2. Light green sector on $y_{11}y_{11}$ leaf. The color of the sector is similar to that of $Y_{11}y_{11}$ leaf and hence the interpretation that these sectors originate by mutation of $y_{11} \rightarrow Y_{11}$. Only certain chemicals can induce sectoring on $y_{11}y_{11}$ leaves.

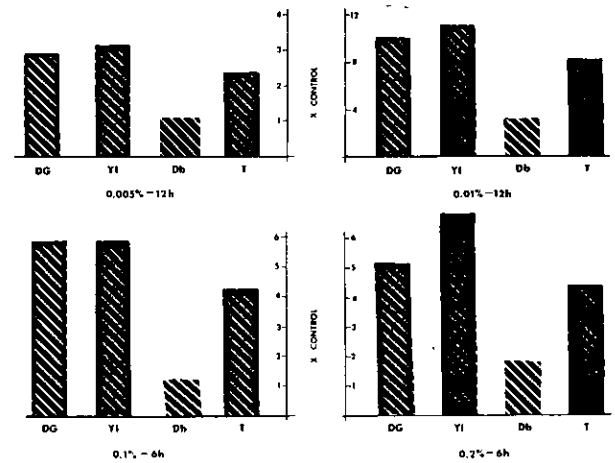

FIGURE 3. Data on the absolute frequency of spots on the leaves of *Glycine* treated with aqueous solutions of 0.005, 0.01, 0.1, and 0.2% NaN_3. There is up to 10-fold increase over control in the frequency of dark green and yellow spots, but the doubles increase only moderately, if at all.

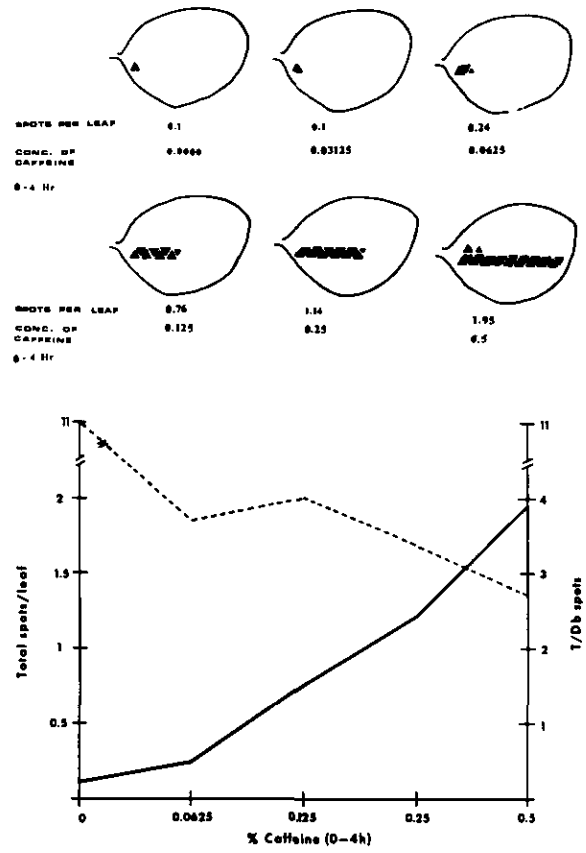

FIGURE 4. Plots of (*a*) the data on total spot frequency on the leaves of *Glycine max* treated with caffeine; (*b*) relationship between (—) total and (--) twin spot. A sharp increase in the relative frequency of twins is apparent.

starting point. As seen in Figure 3, the chemical is capable of producing several-fold increase in the frequency of dark green spots and an equivalent effect on the production of yellows. However, in every sample analyzed, the frequency of twin spots was barely above that found in the control (*28*). The $y_{11}y_{11}$ leaves showed a general lack of effect in the production of light green sectors. However, a few spots, very dark green in color, could be seen on the dark green leaves of $Y_{11}Y_{11}$ plants. These data do not conform with the interpretation that these spots originate due to somatic crossing over, point mutations or segmental losses. Also, NaN_3 has been well tested in plant material to negate its capability in inducing chromosome aberrations (*29*). The tentative conclusion, therefore, remains that this chemical can cause nondisjunction so that $Y_{11}Y_{11}y_{11}$ sectors are dark green and $y_{11}y_{11}Y_{11}$ sectors are near yellow (*28*). Most of the monosomic lines are supposedly incapable of competing with the trisomic ones in growth and are hence lost. We have not been able to find similar action of any other mutagen that we have tested.

Chemicals like caffeine produce a dramatic increase in the frequency of all types of spots (*29*) (Fig. 4), that for twins being most prominent. The mitotic recombinagen mitomycin C (MC) has effects which closely resemble those produced by this oxypurine. The experiments conducted with mitomycin C even at concentrations as low as 3.25 ppm for 24 hr leave no doubt about its effectiveness. The highest relative increase is that for double spots, as if it were the major effect of MC treatment (Fig. 5). Thus it was concluded (*23, 24, 28, 30–32*) that MC brings about complementary segregation for the gene pair $Y_{11}y_{11}$ through the induction of somatic crossing over as its primary effect. The failure of one component or the other of the prod-

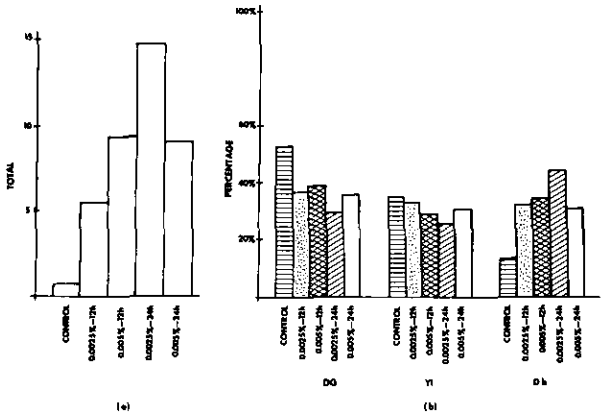

FIGURE 5. Increase in (*a*) the frequency of total spots of $Y_{11}y_{11}$ leaves of *Glycine max*. T219, and (*b*) their respective percentages. The seeds were soaked in aqueous solutions of mitomycin C for 12 or 24 hr. Even though the relative frequency of twin spots is rather low in the control population, the treated material has all three types of spots in nearly equal proportions.

ucts of somatic crossing over or chromosome aberrations like exchanges between nonhomologs, deletions, etc. are considered responsible for single spots. The idea of somatic crossing over having occurred is supported strongly, not only by the type of effect on *Glycine* but also for similar results obtained with tobacco (*9, 10, 33, 34*), *Ustilago*, and *Saccharomyces* (*25*) as well as the quadriradials formed in human (*25, 35*) and plant (*25*) chromosomes after MC treatment. A regression coefficient for the rate of increase of each type of spot on the leaves of *Glycine max* and *Nicotiana tabacum* calculated by Evans and Paddock (*6*) demonstrates that whereas both MC and x-rays are capable of inducing dark green and yellow spots on the leave of heterozygous plants, mitomycin C clearly has advantage over x-rays in the production of twins (Table 2). Similarly, in *Nicotiana (10)*, this chemical has been found to be most effective in producing twin spots when compared with similar effect of radiations and some other chemicals (Table 3).

A third type of effect is produced by agents like ^3H and γ-rays. In this instance, there is an increase in all three types of spots on the heterozygotes (*36*). However, the highlight is an overabundance of yellow spots produced in these treatments (Fig. 6). This is an indication of the agent being able to produce somatic recombination and segmental losses of the chromosome carrying Y_{11}. The Y_{11} deficient cells will produce yellow sectors but those deficient for y_{11} may not be phenotypically different from the $Y_{11}y_{11}$ area surrounding them. These data, and those obtained with NaN_3, fall in line with the quantitative pattern of inheritance for the Y_{11} locus. Ethyl methanesulfonate (*37*), methyl nitrosourea (*38*) dimethylnitrosoamine (*38*) diepoxybutane (*39*), and trenimon (*39*) also produce similar patterns.

The radiations, discussed above, also produce light green sectors on $y_{11}y_{11}$ plants indicating to their effectiveness in inducing point mutations of $y_{11} \rightarrow Y_{11}$ type. These specific locus mutations increase, in general, in frequency with dose and in parallel to the frequency of spots on the light green leaves (Fig. 7). Besides, methyl methanesulfonate (MMS) (*26*), ethyl methanesulfonate (EMS) (*26*), caffeine (*30*), diepoxybutane (*39*), and trenimon (*39*) also produce light green sectors on yellow homozygotes. Another way of looking at comparative effects of some mutagens is shown in Figure 8 reproduced from data obtained with EMS and MMS (*26*).

Table 2. Regression coefficients for the rate of increase of each type of spot on leaves of *Glycine max*.[a]

Spot type	Mitomycin C	X-rays
Dark green	0.01609 log spots/μM MC	0.01700 log spots/R
Yellow	0.01394 log spots/μM MC	0.01673 log spots/R
Double	0.09287 log spots/μM MC	0.00438 log spots/R

[a] Data of Evans and Paddock (*6*).

Table 3. Relative increase in the frequency of twin spots induced by various agents on the leaves of *Nicotiana tabacum*.[a]

Treatment	Increase in twin spot frequency
Caffeine	1.9-fold
Psoralen	2.7-fold
γ-rays	3.1-fold
UV	4.3-fold
Mitomycin C	4.7-fold
Colchicine	Not given

[a] Data of Carlson (*10*).

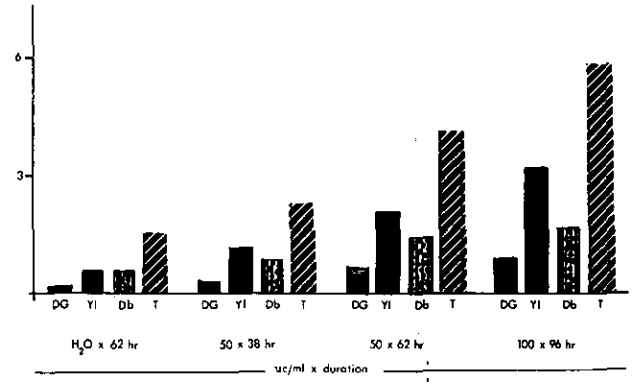

FIGURE 6. Data from an experiment showing increase in the frequency of spots on $Y_{11}y_{11}$ leaves from seed soaked in ^3H_2O. Yellows outnumber the dark green and twin spots in these experiments. This is true also of γ-ray-treated dry or wet seeds.

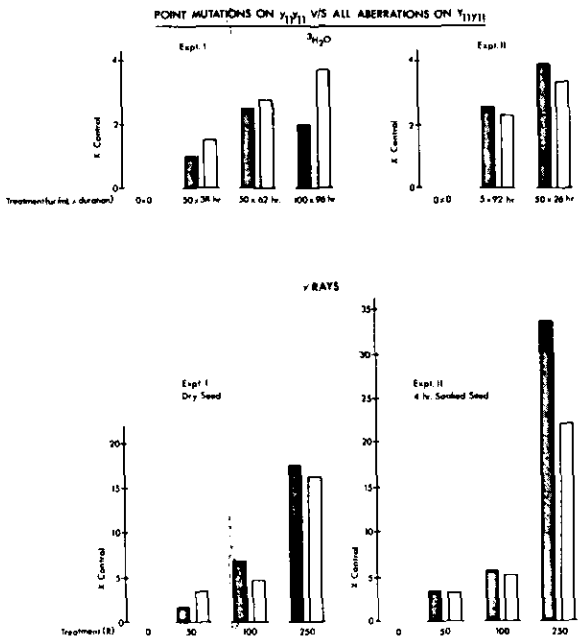

FIGURE 7. Parallel between the frequency of light green spots on $y_{11}y_{11}$ leaves and total spots on $Y_{11}y_{11}$ leaves of *Glycine max* from material treated with ^3H_2O or γ-rays.

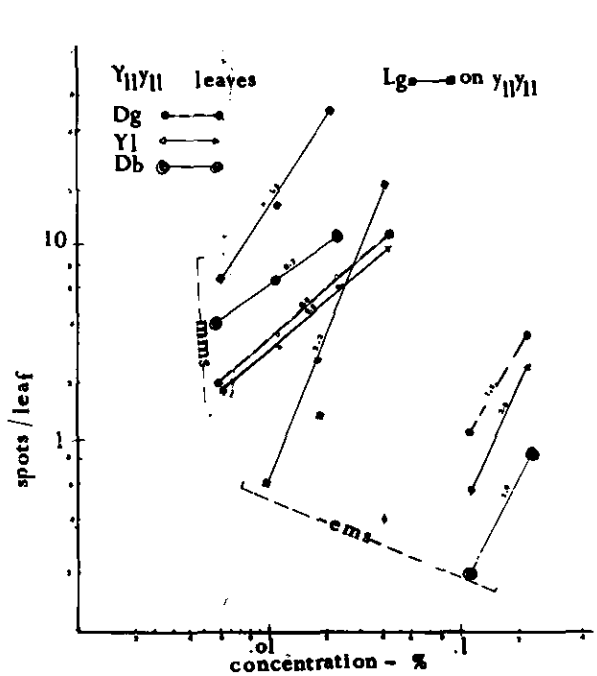

FIGURE 8. Frequency of spots/leaf on *Glycine max* as function of concentration of MMS and EMS. MMS is more effective in the production of all types of spots than EMS, at equivalent concentrations; the increase of twins is more pronounced than those of single spots in case of MMS, but not EMS. Also, notice the slope of the curves for the two chemicals.

Not only are differences in effectiveness of the two alkylating agents in producing spots on the leaves shown but also the effectiveness of the two chemicals is differentiated in production of relatively more twin spots by MMS than EMS. Also, clearcut differences appear between the relative efficacy of the chemicals in producing $Y_{11}y_{11}$ sectors on $y_{11}y_{11}$ plants.

Twin Spots in Other Systems

The occurrence and induction of twins as well as single spots on the heterozygous background is not unique to the *Glycine* system. Similar situations have been found in *Nicotiana tabacum* for the genes a_1, a_2, *t*, and *su* (*9, 10, 33, 34*) and in *Antirrhinum majus* for the genes *inc* and *pal* (Fig. 9) (*2*). In these cases, the frequency of twin spots, as also those of singles, has been increased by administration of solutions of caffeine as in case of *Glycine*. The increases, for example, considering the *Eos, Niv/eos, niv,* heterozygotes has been 53-fold in the frequency of twins and 38-fold in the frequency of singles, for 0.25% caffeine treatment applied for 24 hr. Similarly, increases have been observed by Deshayes and Dulieu (*9*) in the frequency of all types of spots on tobacco leaves treated with γ-rays and mitomycin C. Besides, the well known case of stamen hairs of *Tradescantia* so exhaustively studied by Sparrow, has been found to be highly sensitive to radiations and chemical mutagens. Since the details of this system are being provided in two papers in this symposium, I shall add only that besides other chromosomal aberrations (*15–18*), somatic crossing over has been invoked as a mechanism of production of paired sectors observed on stamen hairs (*19*). Another plant species expressing twin sectors is

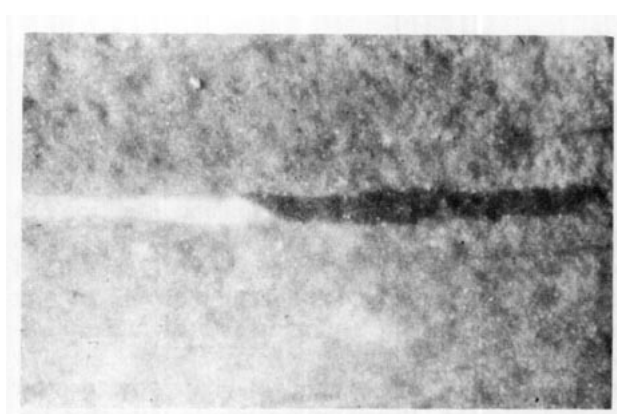

FIGURE 9. A twin spot on petal of *Antirrhinum majus*. The background genotype is *inc eos/Inc eos* and the two components of the sectors are thought to be *inc eos/inc eos* (light) and *Inc eos/Inc eos* (dark).

Petunia hybrida. In this case no spots are observed on the leaves of plants heterozygous for genes a^+/a but can be induced with radiation, e.g., 10,000 R of γ-rays (Vig and Dulieu, unpublished data). Similarly, *Arabidopsis thaliana* and *Lycopersicon esculentum* have been subjected to the induction of twin spots by chemicals. As a matter of fact, all the species listed in Table 1, with the exception of *Hordeum* and *Zea* for which no data for the induction of twin spots exists, show such paired sectors either naturally or under the influence of mutagens.

The frequency of somatic crossing over, or twin spots to be more precise, even though subject to fluctuations from experiment to experiment, may be roughly comparable for organisms widely diverse on evolutionary scale. As Table 4 indicates, the frequency in higher plants and fungi, when tabulated on log scale, is within an order of magnitude, most values being in the neighborhood of 10^{-5} events/cell. When data become available it would be interesting to compare these values with those found in mammals. In case of the housefly, however, sectors are not found in control populations whereas twin spots can be induced with a frequency of 2.7×10^{-2} with 1000 R of x-rays.

Table 4. Comparative rates of spontaneous frequencies of somatic crossing over in various organisms.

Organism	Frequency/cell
Antirrhinum majus	0.96×10^{-5}
Nicotiana tabacum (in vitro)	4.6×10^{-5}
Nicotiana tabacum (in vitro)	6.7×10^{-5}
Nicotiana tabacum (field)	0.77×10^{-5}
Glycine max	12.8×10^{-5}
Tradescantia hirsuticaulis	13.5×10^{-5}
Dictyostelium discoideum	5.0×10^{-5}
Ustilago maydis	5.68×10^{-5}
Saccharomyces cerevisiae	12.0×10^{-5}
Pennicillum chrysogenum	1.08×10^{-5}
Musca domestica	0

Mutagens Requiring Metabolic Activation

Over the last few years, increasing attention has been focused on mutagenic action of chemicals which need metabolic activation. The S9 fraction of the liver homogenate is the common component used to activate promutagens into mutagens. Recent studies have indicated that liver is not the only system which has enzymatic machinery needed for such activation. It was interesting to note that Veleminsky and Gichner (*41*), in 1968, could show the mutagenic activity of some promutagens in the plant systems without requiring the addition of S9 fractions.

In soybean, we treated the seed with aqueous solutions of a nitrosoamine, dimethylnitrosoamine, with concentrations as low as 1.25 ppm for 24 hr. Even at this dose a 2.8-fold increase in the frequency of twin spots, a 2.6-fold increase for dark greens, and 1.7-fold increase for yellows was observed, testifying to the ability of a plant system to activate this chemical. However, one series of treatments using concentrations between 60 ppm and 500 ppm, produced a response indicative of maximal conversion of the chemical into a true mutagen. As Figure 10 shows, such saturation effect was not observed for a related nitrosoamide, methylnitrosourea, which does not require metabolic activation. However, methylnitrosourea seems to be much more toxic in that an adverse effect of leaf expansion, and hence on spot frequency, is observed at 125 ppm doses than that for those of dimethylnitrosoamine which is tolerated by the plant at doses as high as 500 ppm (*38*).

With the data obtained with *Glycine* system and other plants used in the experimental induction of mosaicism, one may list the mutagens found effective in various systems. Table 5 is such a compilation, which in addition indicates whether the frequency of twin spots was less than, equal to, or more than frequency of ½ of both types of single spots. In case of *Glycine*, thus, caffeine, colchicine, methyl methanesulfonate, and mitomycin C are the only agents which have so far been found to in-

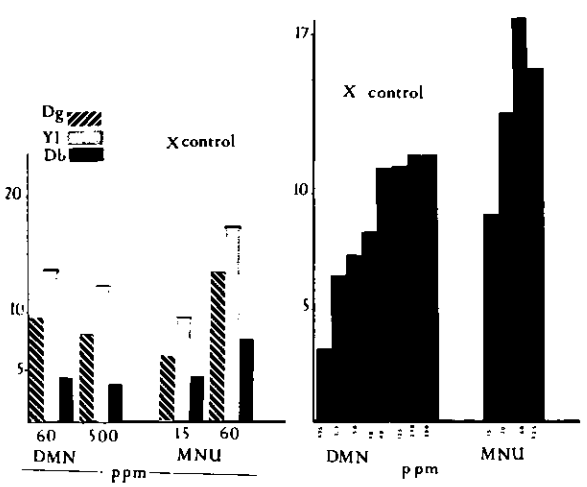

FIGURE 10. Graphs showing (left) the effectiveness of DMN and MNU in producing spots on $Y_{11}y_{11}$ leaves of soybean (both chemicals induce preferential increase in the frequency of yellow spots, doubles show the least increase of all); (right) the saturation effect of DMN at concentrations as low as 60 ppm. MNU appears toxic at concentrations of between 60 and 125 ppm, whereas DMN, perhaps because of its limited conversion to true mutagen is tolerated up to 500 ppm concentration. Bars show total spots × control.

Table 5. Mutagens found effective in increasing the frequency of twin spots in higher plants.

Mutagen	Test system	Twin/single ratio	Reference
Actinomycin D	*Glycine max*	<0.5	Vig (*30*)
Caffeine	*G. max*	>0.5	Vig (*30*)
	Antirrhinum majus	<0.5	Harrison and Carpenter (*42*)
Colchicine	*G. max*	>0.5	Vig (*40*)
	Nicotiana tabacum		Carlson (*9*)
	Lycopersicon esculentum		Ross and Holms (*8*)
Chloramphenicol	*Arabidopsis thaliana*		Ahnstrom et al. (*3*)
1,2-Dibromoethane	*Zea mays*		Nauman et al. (*20*)
Diepoxybutane	*G. max*	<0.5	Vig and Zimmermann (*39*)
Dimethylnitrosoamine	*G. max*	<0.5	Arenaz and Vig (*38*)
Ethyl methanesulfonate	*G. max*	<0.5	Vig et al. (*26*)
	Z. mays		Conger and Carabia (*22*)
	Petunia hybrida		Cornu (*11, 12*)
5-Fluorodeoxyuridine	*G. max*	<0.5	Vig (*30*)
Methyl butanesulfonate	*G. max*		Vig et al. (*26*)
Methyl ethanesulfonate	*G. max*		Vig et al. (*26*)
Methyl methanesulfonate	*G. max*	>0.5	Vig et al. (*26*)
Methyl nitrosoamine	*G. max*	<0.5	Arenaz and Vig (*38*)
Methyl nitrosourea	*A. thaliana*		Ahnstrom et al. (*3*)
Mitomycin C	*G. max*	>0.5	Vig and Paddock (*32*)
Nifurprazinum	*G. max*	<0.5	Vig and Zimmermann (*39*)
Psoralen	*N. tabacum*		Carlson (*10*)
Puromycin	*G. max*	<0.5	Vig (*30*)
Socium azide	*G. max*	<0.5	Vig (*28*)
	Z. mays		Conger (*22*)
	A. thaliana		Ahnstrom et al. (*3*)
Trenimon	*G. max*	<0.5	Vig and Zimmermann (*39*)
γ-rays	*P. hybrida*	<0.5	Vig and Dulieu (unpublished)
	G. max	<0.5	Vig (*36*)
	T. hirsuticaulis		Sparrow (*15*)
			Christianson (*19*) Nauman et al. (*20*); Cuany et al. (*14*); Mericle and Mericle (*16–18*)
	N. tabacum		Carlson (*10*)
^3H_2O	*G. max*	<0.5	Vig (*36*)
UV	*N. tabaccum*		Carlson (*10*)
X-rays	*A. thaliana*		Hirono and Redei (*4*)
	P. hybrida		Cornu (*11, 12*)
	N. tabacum		Evans and Paddock (*6*)
	G. max	<0.5	Evans and Paddock (*6*)

crease the frequency of somatic crossing over producing twin spots more than all other phenomena together working for the production of single spots.

Evidence of Genetic Block by Precursor Analysis of Spots

Harrison and Stickland (*42*) have provided interesting insight into the precise genetic block induced by mutagens on the petals of *Antirrhinum majus*. In this case the *niv* gene blocks all flavonoid synthesis, *inc* blocks the flavanones, *pal* causes a late block after flavonon synthesis, and *eos* locus controls the synthesis of pelargonidin (*eos/eos*) or cyanidins (Eos/-). The insertion of specific precursors into the system can permit the synthesis of pelargonidin or cyanidin. Thus the production of color by dihydrokaempferol in *Pal/-* genotype permits the conclusion that the block was at the *niv* and/or *inc* locus. Administration of dihydiroquercetin can permit synthesis if the block is at *niv* or *inc* loci, but permits further differentiation between *Pal* or pal^{rec} alleles. The administration of dihydroflavone, naringinin, will permit polargonidin development only if the block is at the *niv* locus and also if the *Eos* locus carries the recessive allele, (*eos*). In case of block at *niv* but with *Eos* gene, cyanidin will develop. Since nirengenin cannot lead to production of the end product in an *inc/inc*, neither dihydroquercetin nor naringenin can initiate synthesis in a flower homozygous for *pal* allels. Thus the "aberrant sectors produced in plants heterozygous for *niv*, *inc*, *eos* and *pal* can be analyzed by precursors" (*2*) (Fig. 11). To the best of my knowledge this is the only use of precursors in determining induced variability in plants.

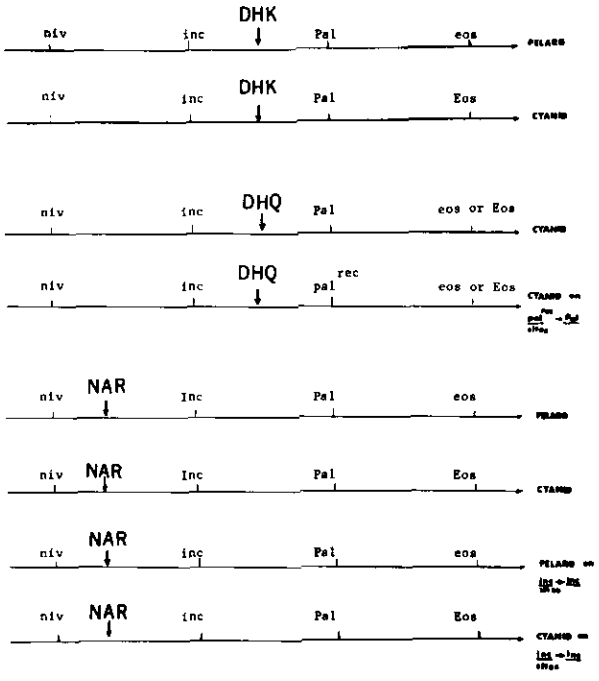

FIGURE 11. Examples of detection of mutant sites in *Antirrhinum majus* by precursor analysis. DHK (dihydrokaempferol) leads to production of pelargonidin (PELARG) in plants with *Pal* and *eos* genes, whereas in *Pal* and *Eos* genotypes the cyanidin (CYANID) develops. DHQ (dihydroquercetol), however, produces cyanidin in *Pal* flowers irrespective of *eos* or *Eos* constitution. In a plant pal^{rec}/pal^{rec} one may detect mutant sectors for $pal^{rec} \rightarrow Pal$ sites by feeding DHK or DHQ to the flower. Similar examples have been sited for NAR (naringenin) which initiates pigment formation after *niv* and before *Inc* gene products come into action.

Controlling Elements

In the case of instabilities resulting from the activity of controlling elements, the frequency of mutational events has not been always affected by exogenous factors like chemical mutagens and x-rays, nor has any effect of pal^{rec} unstable genes on the frequency of somatic crossing over in *Antirrhinum* (*43*) been found. Also in the snapdragon, Harrison and Carpenter could not increase the frequency of instabilities induced by niv^{rec} and pal^{rec} genes by application of EMS, MMS or x-rays (*43*). However, Culella and Gavazzi (*44*) have successfully induced changes in the degree of instability expressed by paramutable R_2^{nc} gene in corn by treating the seeds with x-rays and EMS. This may point to the fact, first reported by Linden in 1963, that the paramutation process is sensitive to physical and chemical agents (*45*). These studies, however, have not been carried on to the extent of making paramutational systems useful in study of environmental mutagenesis.

Interpretations

The interpretation of most data in cytological terms is admittedly only conjectural at this point and would remain so unless techniques are developed for tissue culture of spots, or some flanking markers become available in the species under investigation. Both these conditions have been, however, met with some success in *Nicotiana tabacum*. In such early attempts, Deshayes and Dulieu (*9*) demonstrated the regeneration of mesophyll explants confirming genetic variations involved in the production of spots. Thus variations of nonreciprocal type, i.e., single spots on a_1^+/a_1 background were determined to be due to deletions, point mutations, and gene conversion (*9*). The twins were the result of exchanges between homologous loci as well as between nonhomologs (*33*). On the other hand, Carlson's data (*10*) from plants regenerated from induced spots indicate that 11/12 twin spots resulted from true somatic crossing over, and only one was the result of nondisjunction. An analysis of data from progeny of chimaeric branches in *Arabidopsis thaliana* (*4*) can be interpreted to support the idea of somatic or premeiotic recombination, and Ahnstrom, Natarajan, and Veleminsky (*3*) have attributed induced spots in this species to a phenomenon like somatic recombination. Ample opinion therefore exists that sectors in these systems, as well as those in *Tradescantia* (*19*), originate from somatic crossing over. Additionally, studies in tobacco (*33*) and cotton (*7*) have indicated possible homeologous exchanges giving rise to twin spots.

Single spots may result from more than one cause. Segmental interchanges between homologous or nonhomologous chromosomes, deletions, and additions of the segment or chromosome carrying the gene in question, particularly where the quantitative pattern of inheritance is clear, may result in single exchanges. Another phenomenon involved in origin of such spots may be the failure of one of the original two components of a twin spot from developing, and point mutations. Moizonnier and Cornu (*46*) have provided evidence that mosaicism on petals of *Petunia* results from phenomena like breakage-fusion-bridge cycle. Hagamann's studies (*47*), showing that somatic conversion in tomato may result from localization of *Sulf* locus close to heterochromatin, provide additional evidence of genetic basis for mosaicism. A tabular representation of some interpretations for quantitative genes controlling chlorophyll development has been given elsewhere (*24*).

Advantages of Plant Systems in Study of Environmental Mutagenesis

No one can claim that quantitative data obtained from plants can be utilized for making risk assessments for man. However, one may not neglect the efficiency of plant systems with which they can determine the mutagenic capability of an agent with only a fraction of the cost of such experimentation in animals. More than 99% concordance has been found between plant and animal species regarding their qualitative response to mutagens. This has been demonstrated in a recent NIEHS workshop. A few exceptions, however, among chemicals are the anthracyclines, cytosine arabinoside, and maleic hydrazide, which may affect the genetic system of one kingdom and not the other. One may also need to consider the rapid growth, availability and control of cell stages as well as mitotic, premeiotic, meiotic and post meiotic cells available for treatment. Plants can be vegetatively regenerated, in some instances at least. Somatic mosaicism in such cases has a special place since the advances in tissue culture leading to regeneration of the whole organism may not become possible in animals of routine use, at least in the foreseeable future. Some other advantages, besides those discussed in Introduction, are possible study of storage effects, dosimetry, artificial germination of pollen, etc. In order to study mosaicism as caused by changes in environment, one may look for perennial angiosperms of suitable genotypes and compare results from year to year.

The plant systems, in my opinion, have a place in at least the preliminary screening of new chemicals being poured into the environment. The wonderful ability of plants to activate promutagens may be of great interest, particularly in view of the fact that all of us ultimately have to consume plant materials, many or perhaps all of which have been treated with a variety of agents. From this point alone the study of plant systems including mosaicism should be seriously considered.

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