

## The Action of a Chemical Mutagen on a Mutable System in Maize

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The  $A_1$  locus in maize controlling anthocyanin pigmentation along with a few other loci, shows an interesting behaviour in that while some alleles like  $a_1$ ,  $a^m$  are highly mutable and mutate to the dominant  $A_1$  state in the presence of the non-allelic  $Dt$  gene, others like  $a^{st}$  are highly stable and would not respond to the action of  $Dt$ . The  $a_1-Dt$  system first studied by Rhoades (1938) and more recently by Neuffer (1961) is rather unique as an example of the control of gene action by other genetic elements inasmuch as the action of the  $Dt$  element as a mutator is highly specific being confined to only one locus and to only a few alleles of this locus. This system then appears to provide an opportunity to study several problems of regulation of gene action. For example: What confers specificity to  $Dt$  as a mutator and to the alleles at the  $A_1$  locus the capacity to respond differentially? Can the action of  $Dt$  be altered or copied by genetic elements or nongenetic factors? What is the mechanism underlying stability or conversely high degree of mutability of a genetic element, and particularly to what extent is the molecular organization of a chromosomal segment responsible for such behaviour? Enquiries in these directions would tell us more not only about the regulation of gene action but also the mechanism of gene mutation. Information on any one line of enquiry is likely to offer some clue for the others. To begin with, the simplest problem is to explore if the mutability of the  $Dt$ -responsive alleles can be altered experimentally and particularly through the action of chemicals with some degree of specificity as mutagens. Conversely, it should also be interesting if the stable or non-responsive alleles could be rendered highly mutable through the action of such agents.

The most responsive allele, known yet, is  $a_1^m$ . We record below some results obtained with the  $a_1^m-Dt$  system in response to treatment with maleic hydrazide (MH)—a chemical mutagen in a broad sense and strictly speaking a radiomimetic agent, known to act preferentially on heterochromatin (McLeish 1952). A dual action of the chemical is indicated: (1) induction of point mutation at the  $A_1$  locus (apparent mutation of the recessive to the dominant allele) and (2) possible loss of a chromosomal segment carrying the  $Dt$  element.

### Material and methods

Seeds homozygous for  $a_1^m$ ,  $Dt$  and other genes necessary for plant and aleurone colour ( $A_2$ ,  $C$ ,  $R$ ,  $B$ , and  $Pl$ ) were treated with aqueous MH solution at three different concentrations viz. 50, 100 and 250 parts per million (ppm) for 24 hours. A set of seeds soaked in water served as control. As a result of the mutations of the recessive allele to the dominant  $A_1$  in somatic tissues, streaks of colour appear in internodes, leaf sheaths and other tissues or organs capable of developing anthocyanin pigmentation. The green tissue carrying the non-mutated recessive allele provides a contrasting background for clear revelation of the coloured ( $A_1$ ) spots of varying length. The number and length of the coloured spots were recorded in individual plants under each treatment. Since different 'states' of the  $A_1$  locus may vary in the time of occurrence of responses to  $Dt$  during development (McClintock

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1965), it was considered necessary to observe the frequency and extent of the somatic mutations at least in two stages: (1) at the time of flowering or green stage and (2) at harvest or semi-dry stage. Since the necessary observations could not be taken without injuring the plants, separate lots of plants were studied in two stages. In the green stage internodes and leaf sheaths were examined for the appearance of spots. In the semi-dry stage, only the internodes could be examined, the leaf sheath being too dry for any observation.

### Observations

The relevant data on the action of MH on *Dt*-induced mutability of the  $a_1^m$  allele are contained in Table 1. Considering the average number of

Table 1. The effect of maleic hydrazide on the frequency and length of pigmented spots ( $A_1$ ) in internodes and leaf sheaths of  $a_1^m$ -*DtDt* plants

Treatment	Average No. of spots in internodes per plant (green stage)	Average No. of leaf sheath spots per plant (green stage)	Average No. of internode spots per plant (dry stage)	Length of internode spots* (green stage) in cm	Length of leaf sheath spots* (green stage) in cm	Length of internode spots* (dry stage) in cm
Control	16.0 (8 plants)	16.6 (8 plants)	12.6 (7 plants)	0.48 (0.1-4.5)	0.45 (0.1-3.5)	1.0 (0.1-6.3)
50 ppm MH	9.5 (2 plants)	14.0 (2 plants)	8.0 (11 plants)	1.03 (0.1-9.5)	0.80 (0.1-4.5)	0.53 (0.1-5.2)
100 ppm MH	13.5 (4 plants)	16.5 (4 plants)	9.0 (14 plants)	0.74 (0.1-9.0)	0.30 (0.1-1.3)	0.47 (0.1-6.0)
250 ppm MH	9.75 (4 plants)	13.3 (4 plants)	8.4 (5 plants)	0.44 (0.1-2.7)	0.71 (0.1-15.4)	0.61 (0.1-6.0)

\* Figures inside parentheses represent minimum and maximum values respectively and those without parentheses (upper ones) represent average values.

coloured spots per plant either in internodes or in leaf sheaths, there is an apparent decrease in the frequency of mutations as a result of treatment with MH at any of the three concentrations. This is true in the green as well as the semi-dry stage. It may be noted that irrespective of the treatment, the number of spots in the leaf sheath is higher than that on the internodes. If the average length of the coloured spots is taken into consideration, a different picture would emerge. Observations in the late stage indicate a reduction in the length of spots in the MH-treated series. In the earlier green stage, however, the average length of spot in some of the treatments (e.g. 50 ppm MH) is found to be much higher than that in the control. It will be seen from the Table that with the treatment of 50 ppm MH, the maximum length of the internode spot is 9.5 cm i.e. more than twice the maximum length in any control plant. Under the same concentration of MH, the maximum length of a leaf sheath spot is 4.5 cm i.e. about one and half times as long as the longest spot on an untreated plant. Thus the effect of the chemical on the length of the spot is more pronounced in the internodes than the leaf sheaths. Rather exceptionally, the longest spot (15.4 cm i.e. about four and half times

the maximum length in the control plants) is observed in the leaf sheath of a plant in the 250 ppm MH series.

It should be mentioned here that observation in the semi-dry stage was rendered somewhat difficult because of (1) slight fading of the anthocyanin pigment and (2) discolouration of the green background with the consequent loss of contrast for the spots. This is partly reflected in the data by the invariably low frequency of spots counted in the dry stage in the control as well as the MH-treated series. Hence, the data on the semi-dry plants may appear less reliable to base any conclusion. Nevertheless, the reduction in the number of spots can be observed in the MH-treated series in both green and dry stages. It is thus indicated that inference about the effect to treatment can be drawn at any of the two stages, if the number of spots is taken as the criterion. On the other hand, the effect of MH on the length of spots show different trends in the two stages. In view of the difficulties during observation, the data on this aspect in the semi-dry stage may be less reliable. Hence inference based on length should preferably be limited to the green stage.

Another point of interest can be noted from the data in Table 1. A comparison of the frequencies of spots on the two kinds of organs of the same plants would reveal that while the frequency in the leaf sheath is but slightly higher than that in the internode of untreated plants, the difference between the frequencies in the two organs in the MH-treatments is very striking. Viewing the situation in another way, it would appear that the effect of MH in reducing the number of spots is better manifested in the internodes rather than the leaf sheaths. As discussed earlier, the second effect of MH namely increasing the length of spots is also better manifested in the internodes in the green stage, than in the leaf sheaths.

### Discussion

Two distinct effects were produced by the treatment of seeds with MH: (1) decrease in the number of coloured spots (of  $A_1$  phenotype), and (2) increase in the average length of spots clearly observable in the green stage, MH is known to induce chromosome breaks preferentially in the heterochromatic segments (McLeish 1952). The occurrence of more frequent breaks leading to the elimination of the  $Dt$  gene in the somatic tissue of plants in the MH series may be responsible for the apparent reduced frequency of mutations of the mutable allele to the dominant state. This is likely because the  $Dt$  locus is situated in or adjacent to the heterochromatic knob on the short arm of chromosome 9 (Rhoades 1955). The second effect of MH may arise from a different event. The length of the spot is indicative of the time of occurrence of the somatic mutation ( $a^m$  to  $A_1$ ) in course of development of the plant. The greater the length, the earlier must be the occurrence of these mutations. It is now important to imagine that the number of cells in the

early stage is much smaller than that in the later stages. In that case, mutations in the early stage would also mean that there is an actual increase in the frequency of mutations. This effect, namely increase in the frequency of early mutations (manifested as longer spots) may be due to one or both of the two causes: (1) a direct action of the chemical on the  $A_1$  locus (i.e. the  $a^m$  gene); and (2) an indirect action resulting from a stimulation of the  $Dt$  element as a mutator. The first possibility implies an effect of MH on gene mutation. That MH can induce gene mutations has been indicated in a recent study in rice, *Oryza sativa* (Mohapatra and Sinha 1967 and unpublished). It is not unlikely that such apparent mutation may also involve extra-genic changes or minute chromosomal aberrations rather than intragenic ones as contended by Rhoades (1959). The second possibility of the stimulation of  $Dt$  implies a physiological effect not involving any direct change in the genetic material induced by MH. Distinguishing between these possibilities would certainly lead to a more clear understanding of the action of chemical mutagens besides control of gene action during development.

Other implications apart, the dual action of MH as indicated here appears to be of some practical interest. The  $a^m-Dt$  combination can be used as a suitable system, that would help distinguishing between the two kinds of genetic effects caused by mutagens: 1) gross structural changes in chromosomes and 2) gene or point mutations. This distinction should be useful in studies of chemical mutagenesis, particularly in investigations aimed at determining experimental conditions that would favour one kind of genetic change or the other. Studies of the variables affecting the action of MH may help to increase the efficacy of this chemical as a mutagen. From the present study, it appears that 50 ppm MH causes more mutations and 250 ppm MH induces more aberrations than other concentration.

### Summary

The mutational responses of the  $A_1-Dt$  system in maize to a chemical mutagen, namely maleic hydrazide (MH) was studied in material homozygous for the highly mutable ( $Dt$ -responsive)  $a^m$  allele, as a part of a comprehensive study aimed at understanding factors concerned in the stability (or conversely high mutability) of genetic elements.

A two-fold action of the mutagen could be recognized: (1) one manifested in a reduction in the number of coloured ( $A_1$ ) spots—each presumably resulting from a somatic mutation of  $a^m$  to  $A_1$  in the presence of the non-allelic  $Dt$  gene; and (2) the other expressed as an increase in the average as well as maximum length of coloured spots. The former is probably due to the loss of the  $Dt$  gene (chromosomal aberration) and the latter due to more frequent early somatic mutations.

The use of the  $a_1^m-Dt$  system for distinguishing between different effects of a mutagen was suggested.

### References

- McClintock, B. 1965. The control of gene action in maize. Brookhaven Symp. Biol. 18: 162-184.
- McLeish, J. 1952. The action of maleic hydrazide in *Vicia*. Heredity 6 (Suppl): 125.
- Nuffer, M. G. 1961. Mutation studies at the  $A_1$  locus in maize. I. A mutable allele controlled by *Dt*. Genetics 46: 625-640.
- Rhoades, M. M. 1938. Effect of the *Dt* gene on the mutability of the  $a_1$  allele in maize. Genetics 23: 377-397.
- 1955. The cytogenetics of maize. In "Corn and Corn Improvement", Ed. G. F. Sprague. Chapter 4: 123-220. Acad. Press, Inc. N.Y.
- 1959. The cytogenetics of maize. Jessup lecture at Columbia University for 1958. (Cited in Taylor, J. H. 1959. The organization and duplication of genetic material. Proc. 10th Intern. Congr. Genet. Montreal. 1: 63-78).
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