AN EMS-SENSITIVITY FACTOR IN MAIZE CONDITIONING ALBINO LEAF STRIPES*

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ABSTRACT

A description is given for a recessive EMS-sensitivity factor conditioning albino leaf stripes. In the homozygous condition, there is low frequency of spontaneous leaf stripes. EMS, depending on the concentration, increases markedly the frequency of the stripes in 100% of the treated plants. The effects of EMS, given to the seeds, starts to appear from the 6th to 7th leaf and persists in all the leaves throughout the life cycle of the M_1 plants. Only about 20–25% of the M_2 plants showed some variegation. These variegated M_2 plants were not distributed at random among the different ear-to-row M_2 rows.

THE chemical mutagen ethyl methanesulfonate (EMS) is at the present the most widely used in crop plants. It is a powerful mutagen known for the induction of chlorophyll mutations in higher plants (GAUL *et al.* 1966). STADLER (1930) was the first to show that in maize mutations induced by seed irradiation appeared as sectors in the M_1 plants. These sectors were considered as somatic mutations or chromosomal abernations and did not draw much research effort. They were used, for example, by ANDERSON *et al* (1949) and by STEIN and STEFFENSON (1959) to study the ontogeny of organs in the M_1 plans of maize.

High rates of M1-induced sectors were reported in maize by MOULI and NOTANI (1968), BHARATHI and REDDY (1969) and by TING and DOUGALL (1969). These rates are higher than would be expected from what we know about mutation rates at specific gene loci. High rates of M_1-induced sectors were also found in other crops (e.g., SAVIN, SWAMINATHAN and SHARMA 1968; in barley; and SHAMA RAO and SEARS 1964, in wheat).

The present report describes a case of an extremely high rate of EMS-induced M_1 albino stripes. The EMS-susceptibility is controlled by a single chromosomal recessive allele in a specific inbred line of maize.

MATERIALS AND METHODS

Three different inbred lines of maize having low (AD-7), normal (AD-1) and high (AD-19) activity of alcohol dehydrogenase (EFRON 1970, 1973a), were initially treated with 0.005 and

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0.01 M EMS. Albino stripes were found in all treated plants of the inbred line AD-1. This line was used for a more detailed study.

Inheritance studies: All possible M_1 plants from the inbred line AD-1 were self-pollinated (91 and 205 good ears were obtained from the 0.01 and 0.005 M EMS treatments, respectively). In the following year, 15 seeds from each ear were planted in the field. Self-pollinated seeds from untreated plants were grown as a control. The plants were scored for the appearance of albino stripes three times: at the seedling stage (6–7 leaves), 30 days after germination, and at the tasseling stage (65 days after germination). Other M_2 chlorophyll mutations were scored at the seedling stage and the degree of variegation was scored at the tasseling stage.

Effect of EMS concentration: Seven different EMS concentrations (0 and 0.001, 0.0005, 0.001, 0.005, 0.01, 0.1 M) were applied to seeds of AD-1. The treated seeds and untreated control seeds were planted in a greenhouse in four replications. The plants were harvested 33 days after germination (the 0.1 M-treated seeds did not germinate) and scored for the degree of variegation and some other growth characteristics.

Genetic studies: Seeds of the inbred lines AD-1 and AD-7, their two reciprocal F_1 hybrids, and all possible F_2 and B.C. progenies, were treated with 0.01 M EMS, planted in the field and classified for their variegation 45 days after germination.

EMS treatment: The seeds were treated with EMS solutions of the various concentrations for 16 hours at 25°. Then, they were washed for 4 hours in tap water and planted wet in the field or greenhouse.

Degree of variegation: The degree of variegation was scored on a numerical basis (from 0 to 10 in the greenhouse and from 0 to 5 in the inheritance studies), according to the number of stripes per leaf and the number of leaves showing albino stripes. Thus, zero indicates plants without any stripes, 1, a plant showing only one stripe on one leaf, and 10, a plant in which all leaves showed at least five albino stripes.

RESULTS

An EMS mutation study was carried out with three inbred lines of maize having different activity levels of alcohol dehydrogenase (ADH). Two concentrations (0.01 and 0.005 M) of EMS were applied. Every one of the M_1 plants of the inbred line AD-1 showed albino stripes on the leaves, while the other two lines (AD-7 and AD-19) did not show any variegation. At this stage only general observations were made. The number of albino stripes and their width varied from plant to plant and also from leaf to leaf (Figure 1). The albino

FIGURE 1.—EMS-induced albino stripes in plants of the AD-1 inbred line of maize.

stripes were found starting from the 6th–7th leaf on, including the uppermost leaf and the outer leaves of the husk. There was an increase in the degree of variegation and especially in the width of the stripes as the plant grew. Usually, the M_1 plants treated with 0.01 M EMS were more variegated than those treated with 0.005 M.

Effect of EMS concentration: A more detailed study of the effect of EMS concentration was carried out in the greenhouse (Table 1). As observed before, there were no albino stripes of the first 4–5 leaves in any treatments. Twenty-eight percent of the control plants showed albino stripes. However, in almost all of the plants there was only a single very narrow stripe on only one leaf. Therefore, the degree of variegation in the control was 2.95. The number of control plants with albino stripes which were found in this experiment was higher than that observed in the heritability studies under field conditions (Table 2). The control plants in the greenhouse were examined more thoroughly. Therefore, it is possible that a number of plants with stripes were not counted in the field experiment. However, it is also possible that since the control seeds were washed together with the EMS-treated seeds, there was some effect of EMS in the control plants also.

The induction of albino stripes was clearly EMS-concentration-dependent (Table 1). As the concentration of EMS increased, more plants showed albino stripes and a higher degree of variegation. The degree of variegation is only an estimated number of the events which had occurred leading to the appearance of albino stripes. However, this is a much better estimate than the percent of plants with stripes.

Germination was affected only at the highest EMS concentration. Plant growth was slightly affected at 1×10^{-2} M EMS and to a much larger extent at 5×10^{-2}

TABLE 1

Effect of EMS concentration on the appearance of albino stripes, percent of germination, dry weight and width of the sixth leaf in the inbred AD-1 of maize

EMS concentration (M)	Percent from control*			Albino stripes	
	Germination	Dry wt/ plant	Width of 6th leaf	Percent plants† with stripes	Degree of variegation
0	100.0	100.0	100.0	28	0.3
0.0001	97.4	102.1	102.5	40	0.7
0.0005	102.5	99.1	94.9	53	1.0
0.001	102.5	96.6	85.7	73	1.9
0.005	92.3	95.8	79.7	98	3.8
0.01	102.5	84.0	79.7	100	5.5
0.05	100.0	22.6	40.9	100	9.2
0.1	0.0	—	—	—	—

* Fifteen seeds were planted in each of four replications. After germination the replications were thinned to ten plants each. Measurements were taken from all plants.

† Plants with only one narrow albino stripe on one leaf were also considered as having albino stripes.

TABLE 2

Frequency of M_2 plants with stripes during the growth of the plants

Plant age	Frequency of plants with stripes (%) Control	EMS-treated M_2 plants 0.005 M	0.01 M
Seedling	0.8	0.45	0.72
30 days	5.1	4.2	6.4
65 days	8.4	23.5	22.8

M. The width of the leaves is a more sensitive seedling measure of EMS sensitivity (EFRON 1973b) and it was already reduced at 1×10^{-3} M EMS.

Albino striping in the M_2: In the inheritance studies, 91 and 205 selfed ear to row M_2 rows were planted from the 0.01 M and 0.005 M-EMS treatments, respectively. The number of plants with stripes was increased from the seedling stage to 65 days after germination in both the control and the M_2 plants (Table 2). At the seedling stage and 30 days after germination there were no differences between the control and the M_2 plants. However, at 65 days there were significantly more plants with albino stripes in the M_2 plants. In the control, even though there were more plants with albino stripes at 65 days after germination, the average number of stripes per leaf was more or less constant. At the seedling stage only the upper 1–2 leaves could show the stripes, while at 65 days there were about 12 leaves that could show stripes. A more detailed study has to be done in the future, but it is estimated that the spontaneous rate of stripes per loaf is about 0.8%.

At 65 days the difference between the M_2 and the control plants was not only in the number of plants with stripes but also in the degree of variegation (Table 3). In the M_2 generation the number of stripes was smaller than that obtained in the M_1. Therefore, the plants were classified only on a numerical scale, from 0 to 5. In the control, most of the plants (92.6%) had one or a few stripes only; none of them was classified as 4 or 5. On the other hand, in the M_2 there were fewer plants with values of 1 and 2 and more plants with values of 3, 4 and 5.

TABLE 3

Distribution of the degree of variegation among the plants with albino stripes in the M_2 and the control plants 65 days after germination

Degress of variegation	Frequency (%) from plants with albino stripes Control	0.005 M EMS M_2 plants	0.01 M EMS M_2 plants
1	66.6	57.4	43.1
2	26.0	26.5	26.9
3	7.4	10.4	15.8
4	—	4.7	8.2
5	—	1.0	6.1

TABLE 4

Distribution of the pooled degree of variegation among the individual selfed ear to row M_2 rows 65 days after germination

Pooled degree* of variegation	Frequency of M_2 rows (%) 0.005 M	0.01 M
0–10	56.7	54.0
11–20	35.3	29.6
21–30	5.0	6.8
31–40	3.0	6.8
41	—	2.8

* Pooled degree of variegation = the sum of the degree of variegation values given to all plants in the row.

TABLE 5

Number and type of induced EMS chlorophyll "true" mutation

Mutant	Number of segregating M_2 rows: Control	0.005 M	0.01 M
Albino	—	1	1
Yellow leaves	—	2	3
Light green leaves	—	1	1
Yellow stripes	—	7	—
Total	—	11	5

TABLE 6

*Segregation of plants with EMS-induced albino leaf stripes in crosses of the AD-1 EMS-sensitive line and AD-7**

Pedigree	Number of plants: With stripes	Without stripes	Total	χ^2	P
AD-1	44	—	44		
AD-7	—	48	48		
AD-1 × AD-7	—	47	47		
AD-7 × AD-1	—	48	48		
(AD-1×AD-7) × AD-7	—	70	70		
(AD-7×AD-1) × AD-7	—	77	77		
(AD-1×AD-7) × AD-1	32	38	70	.51†	.50–.30
(AD-7×AD-1) × AD-1	38	44	82	.44†	.70–.50
(AD-1×AD-7) (AD-1×AD-7)	72	176	248	2.22‡	.20–.10
(AD-7×AD-1) (AD-7×AD-1)	62	170	232	.29‡	.70–.50

* All seeds were treated with 0.01 M EMS and tested 45 days after germination.
† Tested for goodness of fit to 1:1 ratio.
‡ Tested for goodness of fit 3:1 ratio.

This tendency was greater in the 0.01-M than in the 0.005-M-EMS treated M_2 plants.

The M_2 plants with albino stripes were not distributed randomly between rows. The range of plants with stripes was 0–10 per row. The same is true for the degree of variegation. Table 4 summarizes the distribution of values obtained for each row by summing the values given to all plants in the row.

The EMS treatment was also effective in producing "true mutations." Sixteen segregating rows (5.4%) of chlorophyll mutants were found (Table 5).

The genetics of EMS susceptibility: The genetics of susceptibility to EMS expressed by albino leaf stripes was studied by treating the inbred lines AD-1 and AD-7, their two reciprocal F_2 hybrids, and all possible F_2 and B.C. progenies with 0.01 M EMS. The results (Table 6) show that the susceptibility is due to a recessive allele of a single chromosomal locus, inherited in a simple mendelian fashion.

DISCUSSION

The results presented in this paper clearly indicate that EMS induces a specific variegated phenotype at a specific gene locus presented in inbred AD-1. In a later study (EFRON and BINIAMINY, unpublished) the same response was not found among 100 different inbred lines treated with EMS at a concentration that induces variegation in 100% of the treated AD-1 plants. The EMS sensitivity, conditioning an albino variegated phenotype, is under genetic control of a single recessive allele of a chromosomal locus. In maize there are several factors conditioning albino leaf stripes (NEUFFER, JONES and ZUBER 1968). It is possible that the sensitive target for the EMS effect is one of these known loci. However, it is also possible that the sensitive primary target could be any other locus and the variegated phenotype is due to a secondary effect. Such a possibility was demonstrated for the iojap phenotype of maize. The iojap phenotype is characterized by longitudinal white stripes on the leaves when the gene iojap is in the homozygous recessive condition (JENKINS 1924). RHOADES (1943) has shown that this phenotype may persist in the maternal line even in the absence of the mutant gene. It was suggested (RHOADES 1946) that the *ij* gene induces plastid mutation or the production of an abnormal plasmagene which controls plastid development.

There are five to six leaf primordia in the dry seed of maize (STEIN and STEFFENSEN 1959). These leaves are not affected by the EMS treatment. Stripes started to appear in the 6th or 7th leaf. In these leaves there are usually only one or two narrow stripes and the number of stripes is increased in the succeeding leaves. Therefore, the EMS-directed change probably occurs in undifferentiated meristematic cells. However, once the change has occurred, it is a permanent, irreversible change, passing from cell to cell by mitosis throughout the life cycle of the treated plant. Leaf color is a function of the chlorophyll found in the chloroplast. The autonomous self-replicating state of the plastids in plants is well documented. Thus, it might be assumed that EMS causes an irreversible change in the plastids. But, plastids that have reached a certain stage of development (those found in the embryo's leaf primordia) are not subjected to this type of change. As in the case of the iojap gene (RHOADES 1946), it is not known whether EMS induces a direct change in the plastid or an abnormal plasmagene or nuclear gene controlling plastid development.

The generative tissue of the tassel of the main stalk in maize is derived from 7 to 8 cells present in the apex of the dry seed (ANDERSON *et al.* 1949). It is assumed that the same is true for the generative tissue of the ear. This should be taken into consideration in the interpretation of the results obtained for the M_2 plants. The EMS-induced stripes are not transmitted as such to the next generation, but there is a residual effect of the EMS in the M_2 plants. About 20–25% of the M_2 plants showed some degree of variegation (Table 2), but on a smaller scale than the M_1 plants (Table 3). That not all the cells in the embryo were affected by EMS is evident from the variegated pattern obtained. The same is probably true for the primordial cells of the generative tissues. Thus, it is expected that in some of the M_2 plants, none of the generative primordial cells was affected by the EMS. The progeny of these plants will not show any variegation. The progeny of the other plants are expected to show variation in the number of variegated plants. This depends on the number of the generative primordial cells affected by EMS, and the mode of inheritance (cytoplasmic or nuclear). In addition, variation might be affected by selection against the EMS-induced cells during the development of the generative tissues. This type of variation was found among the M_2 rows (Table 4). Although the mode of inheritance is not completely understood at present, the results obtained in the M_2 showed that the acquired variegation is at least partially inherited.

The results obtained in this study are not readily explained in conventional terms. From what we know about the mutation rates usually induced by mutagens, it is highly improbable to obtain such a high rate of true mutation (Table 1). This is especially true since the change is from a dominant to a recessive phenotype. A simultaneous change would have to occur in both chromosomes to manifest such a change in the first generation. Therefore, we would like to propose possible explanations for the results obtained.

It is possible that the EMS-sensitive factor behaves like, and even might be allelic to, the *ij* allele (RHOADES 1943, 1946). However, while homozygous *ij/ij* genotype is sufficient for the production of irreversible stable change in the chloroplasts or the plasmagene, the effect of the EMS-sensitive factor is expressed phenotypically only rarely (spontaneous leaf stripes). According to this hypothesis, EMS "magnifies" the action of the EMS-sensitive factor. As mentioned before, it is possible that this factor is allelic to the iojap allele. But, it also may be an allele of any other locus controlling plastid structure or development.

The second possible explanation is that the EMS-sensitive factor is an unstable allele. Locus-specific genetic instabilities have been reported in a variety of eukaryotic organisms. They were recently reviewed by GREEN (1973) for *Drosophila* and by FINCHAM (1973) for plants. In maize, several systems of mutable or unstable loci have been described (for review see MCCLINTOCK 1965 and PETERSON 1970). The behavior of the EMS-sensitive factor is reminiscent of the above gene control systems. Therefore, the results obtained might fit into the category of unstable alleles. The presence of spontaneous albino stripes favors this explanation. However, from the data available this possibility cannot be accepted or rejected at present.

The paramutation phenomenon (BRINK 1964) which may be described as allele-specific, directed-phase changes, is a phenomenon related to the category of genetic instability. The finding that the *R'* alleles are highly sensitive to heritable changes in response to treatment of heterozygous *R*/*R*-st seeds with DES and EMS (AXTELL and BRINK 1967) may also support this hypothesis of an unstable allele. X-rays were also found to be an artificial paramutagen (SHIH and BRINK 1970). It is of interest to note that the *Ej* allele (extension of japonica) results in extreme expression of *ij*, *sr2* and certain other striped leaf mutants (NEUFFER, JONES and ZUBER 1968). It is usually found with recessive aleurone color alleles of the *R* locus. According to STADLER (see NEUFFER, JONES and ZUBER 1968), it may be an expression of the *r* allele.

Recently it was found that the element which suppresses iojap and striate-2 chlorophyll variegation is closely linked but separable from the *R* locus by recombination (KERMICLE, unpublished). The AD-1 line may be homozygous recessive for either japonica stripe, iojap stripe, striate-2 or some unknown locus conditioning chlorophyll variegation, but expression of the variegated phenotype may be suppressed in the presence of an inhibitor located close to the *R* locus. This presumes that AD-1 is homozygous for a chlorophyll variegation gene which is repressed. Treatments with EMS induced de-repression in either the chlorophyll variegation gene directly or by inducing de-repression of an inhibitor element at some other site in the genome (possibly near the *R* locus). It should be noted that the AD-1, AD-7 and AD-19 lines are all homozygous for the *r* allele (colorless seeds and anthers).

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