

Preliminary analysis of the linkage data for chromosome four indicates control of pith abscission ( $ab^P$ ) on the short arm and control of rind abscission ( $ab^R$ ) on the long arm. Although the genes controlling these two areas of abscission may be 50 crossover units or more apart, they tend to be inherited together in hybrids of corn four with Nobogame teosinte four because of a reduction in crossing over in the Su-G1<sub>3</sub> region. Crossing over is normal in similar hybrids with the fourth chromosome from Florida teosinte, a variety of the primitive Guatemalan teosinte, Jutiapa. Final analysis of the linkage data awaits completion of the tedious sawing of longitudinal sections through some 1000 highly lignified corn cobs.

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1. Chemical mutagenesis following treatments at different developmental stages.

The study of the effectiveness of chemical mutagens, administered at different stages of ontogeny, upon induction of mutations might bear useful information of both theoretical and practical value. We chose for this analysis an alkylating agent, ethyl methane sulphonate (EMS), whose mutagenic power has been established in many organisms.

The following treatments were performed:

1. Seeds: soaking for 24 hr. in the mutagen solution at  $23 \pm 2^\circ$  C
2. Seedlings: immersion of the primary root in the mutagen solution after removal of its distal portion to insure better up-take
3. Plants at the time of male meiosis: injection of 10 cc of EMS solution into a Pasteur pipette with its tip inserted into the plant stem
4. Pollen grains: as in previous stage

For each treatment a freshly prepared 2% EMS solution adjusted to pH7 with phosphate buffer was used.

The following mating scheme was adopted: Control and treated sibs, homozygous for  $R_2^{nc}$  Pr Sh and Y, were reciprocally crossed with a corresponding multiple recessive stock ( $r^g$  pr sh y). The resulting ears were then scored for production of sectors of seeds with nonparental phenotype. If the dominant markers, Y, Sh and Pr, are lost or mutate the recessive character may appear, while any change leading to resumption of R function is registered by production of pigment in the aleurone layer of the endosperm. The effect of EMS upon R expression will be considered elsewhere; results from studies with the other three genes are presented in Table 1.

Table 1

Frequency of mutations induced by EMS at specific loci (y, pr and sh).

Plant stage treated	n (a)	Kernels scored	Mutants	Fr. ( $\times 10^{-3}$ )
Control	102	14653	0	---
Seeds	61	12021	2 <sup>(b)</sup>	32.78
Seedlings	83	8431	1 <sup>(c)</sup>	12.04
Premeiotic	37	5032	0	---
Pollen grains	28	7144	0	---

(a) total number of ears and/or tassels

(b) 1 sh and 1 pr

(c) y

From each M1 ear, three or four kernels were taken and planted to measure the frequency of mutation in M2 and M3 generations. Kernels were removed from different areas of each M1 ear to avoid a duplication of any mutation, on the assumption that induced mutations transmitted through the female gametophyte tend to occur in clusters. The M2 ears were then scored for segregation of "endosperm mutants". This category includes aborted and small seeds, defective endosperm and viable mutants with abnormal endosperm morphology. From each ear a sample of 50 seeds was germinated in the sand-bench. The resulting seedlings were scored for the appearance of mutants

Table 2  
 Frequency (%) of endosperm and seedling mutation as measured  
 in M2 and M3 progenies.

Plant stage treated	n	Mutations affecting:									Total
		Endosperm			Chlorophyll synthesis			Seedling morphology			
		(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	
control	148	.00	---	---	.00	---	---	1.35	1.35	---	1.35
seed	109	26.60	13.76	12.84	11.92	11.00	.92	17.43	6.42	11.01	55.96
seedling	99	15.15	3.03	12.12	8.08	8.08	---	2.02	1.01	1.01	25.25
premeiotic	46	.00	---	---	.00	---	---	2.17	---	2.17	2.17
pollen grain	104	.00	---	---	.96	.96	---	.00	---	---	.96

(a) total; (b) 3 : 1 segregation; (c) segregation other than 3 : 1

affecting chlorophyll synthesis or seedling development. The results obtained are presented in Table 2.

In the table each group of mutants has been subdivided, by means of a chi-square test, into two subgroups, i.e., those fitting a 3:1 ratio, presumably point mutants, and those with a significant shortage of segregating mutants, presumably small intercalary deletions. This subdivision shows that, while both endosperm and seedling mutants are variously distributed among the two subgroups, the majority of chlorophyll mutants fit the 3:1 segregation. Furthermore, as in the case of the mutagenesis at specific loci (Table 1), the stage most sensitive to EMS treatment appears to be the seed, followed by seedling, while later treatments seem to be ineffective.

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## 2. A test of the response of some chlorophyll mutants to different temperature and nutrients.

Mutants with identified blocks in the synthesis of an essential metabolite are very rare in higher plants (cf. Nelson, 1967 and Redei, 1970 for a review). Maize offers a large series of mutants well characterized genetically but not yet investigated from the point of view of their metabolic effect. These mutants, being involved in the control of essential functions like chlorophyll and chloroplast synthesis, plant development, and morphogenesis, represent good material for the analysis of the chain of events linking the gene to its phenotypic effect.

Among all those available, we chose the "chlorophyll mutants", i.e., mutants characterized by a more or less strong reduction in chlorophyll content or by its complete absence. We made a test of the response of these mutants to exogenous sources of nutrients and to different temperature. The test consisted in growing excised mutant embryos under sterile conditions in testtubes containing either mineral or supplemented media at two temperature levels (20 and 30° C) in a growth chamber under continuous light (approximately 300 foot candles). The composition of the mineral medium (M.M.) in mg/l bidistilled water is as follows:  $\text{NH}_4\text{NO}_3$  600;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  400;  $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  400;  $\text{KH}_2\text{PO}_4$  400;  $\text{K}_2\text{HPO}_4$  160;  $\text{FeC}_6\text{H}_5\text{O}_7 \cdot 3\text{H}_2\text{O}$  6.