

be made chromatographically and spectrophotometrically if one is interested in the actual identity of the pigments.

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2. Plastid pigments in white-1 luteus-1 ( $w_1/w_1$   $l_1/l_1$ ) seedlings.

Seedlings of the genotype  $w_1/w_1$   $l_1/l_1$  are of interest in that they would probably be scored as xantha rather than albina by most observers. Extracted pigments from greenhouse-grown  $w_1/w_1$   $l_1/l_1$  seedlings yielded at least seven detectable plastid pigments when chromatographed on and eluted from sucrose (powdered confectioners sugar) columns. Tentative identification of the pigments included chlorophyll  $a$ ,  $\beta$ -carotene, lutein, violoxanthin and neoxanthin but quantities at hand were insufficient for spectral confirmation of the separated pigments.

Pigments detected in leaves of  $w_1/w_1$   $l_1/l_1$  seedlings were 10% or less than quantities found in normally green maize leaves at the same age, six days after emergence. Of particular interest is that a similar quantity of leaf tissue (0.5 g.) from homozygous  $w_1$  seedlings with dominant alleles at the  $l_1$  locus did not provide sufficient pigments for separation using the same chromatographic technique.

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1. Chemical mutagens in mineral oil very effective on corn pollen.

For a number of technical reasons, chemical mutagens are generally ineffective when applied to corn pollen. However, when mineral oil (white domestic paraffin oil) is used as a carrier for the pollen (Coe MNL 40:108, 41:139) effective concentrations can be brought in direct contact with the pollen grains. The results with ethyl methanesulfonate (EMS) and nitroso guanidine (NG) have been especially impressive.

The procedure with EMS is as follows: Prepare a solution of .01 to .1% EMS in mineral oil. Place fresh pollen in a shell vial and add 10 times its volume of treatment solution. Stir immediately with a #10 camel hair brush. Wait 3-5 minutes to begin pollination. Pollinate by stirring pollen, then applying moderate amounts of the mixture to the silks with the brush. With EMS it is necessary to proceed rapidly as the pollen will be killed in less than 20 minutes. Extreme caution should be used to protect handlers as this is a dangerous chemical especially in mineral oil, which will not wash off easily. Disposable gloves, eye protection, and sanitation are vital.

The same procedure is used with NG except that it is in crystalline form and highly insoluble in mineral oil. Place a small quantity of crystals (.4 gram +) in 100 ml of mineral oil. Do not use solvents as they will

kill the pollen immediately. Shake until the crystals are widely dispersed in the oil. Allow to stand for several hours until all crystals have settled out then pour off the solution and use as indicated above. However, in this case begin pollination whenever ready and continue as long as necessary. Pollinations made at 90 minutes give the same excellent results as those made at 3 minutes.

With EMS, selfed progeny from 42 treatments have yielded 334 good endosperm and seedling mutants, including many resembling known mutants.

Both chemicals are effective in producing large numbers of endosperm losses in experiments designed to test for them. When  $\underline{A}^b \underline{Sh}_2 \underline{et}, \underline{Dt}$  pollen was treated with NG and crossed on  $\underline{a}^m \underline{sh}_2 \underline{Et}, \underline{dt}$  silks, the following results were obtained.

Frequency ( $\times 10^{-4}$ ) of loss of Components of the  $\underline{\alpha} \underline{\beta} \underline{Sh} (\underline{A}^b \underline{Sh})$  segment from treatment with nitroso guanidine in mineral oil

Treatment	Population	$\underline{\alpha} \underline{\beta} \underline{Sh}$ (Colorless, shrunken)	$\underline{Sh}$ (Colored, shrunken)	$\underline{\alpha} \underline{\beta}$ (Colorless, normal)	$\underline{\beta}$ (Dilute, normal)
Whole endosperm					
Control	8667	3	0	3	6*
NG	22058	48	4	4	5*
Fractionals 1/8 +					
Control	8667	65	3	13	9*
NG	22058	817	196	130	134

\*Progeny tests will probably eliminate most of these as resulting from exchange between  $\underline{\alpha}$  and  $\underline{\beta}$  at meiosis.

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1. Map location of  $c_2$ .

The placement of  $c_2$  distal to  $\underline{gl}_3$  on chromosome 4 is established by the following data from the cross of  $\underline{+ gl}_3 \underline{c}_2 \times \underline{Tu gl}_3 \underline{+ / + + c}_2$ :