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 ($x10^{-4}$) 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 $\alpha$ and $\beta$ at meiosis.

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1. Map location of $c_2$.

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

| Parental | | Reg. 1 | | Reg. 2 | | Doub. | | T |
|---|---|---|---|---|---|---|---|---|
| 56 | 59 | 13 | 13 | 7 | 9 | 0 | 1 | 161 |
| 118 | | 26 | | 16 | | 1 | | |

$\underline{Tu} - \underline{Gl}_3 = 16.8 \pm 2.9$ $\underline{Gl}_3 - \underline{C}_2 = 10.6 \pm 2.4$ c = 0.35

Two-point data from the cross of $\underline{+}\ \underline{+}/\underline{gl}_3\ \underline{c}_2$ x $\underline{gl}_3\ \underline{c}_2$ give a better estimate of the map distance:

| | | + + | + c | gl + | gl c | T | % |
|---|---|---|---|---|---|---|---|
| $\underline{Gl}_3\ \underline{C}_2$ | CB | 468 | 22 | 28 | 440 | 958 | 5.2 ± 0.7 |

E. H. Coe, Jr.

2. Selective enrichment experiments with pollen.

Tests parallel to those outlined last year (News Letter 41: 139) show some encouraging results. Tests with pollen from a multiple heterozygote for $\underline{bz}_2$, $\underline{a}_1$, $\underline{c}_2$, $\underline{a}_2$ $\underline{pr}$, $\underline{c}_1$ $\underline{bz}_1$, and $\underline{r}$ (8 markers, 5 chromosomes) have been analyzed. Tests with other markers are in progress.

Pollen from the multiple heterozygote (1 ml) was mixed with 4 ml of aqueous medium (modified according to work of Y. H. Chang; 0.35M sucrose plus 1,200 ppm Ca $Cl_2$) and applied with a # 8 or # 9 brush to the silks. Each mixture was used on all 8 recessive testers, one ear each. The medium contained one of two concentration levels of one of 28 different agents. The list below gives the class, identity, and concentration of each agent tested. Ratios are given only for tests in which one or more of the ratios for that marked chromosome were significantly deviant (**1%, *5%, + or - 10%). Three hundred and eight of the 450-odd pollinations yielded sufficient seed for statistical test of the ratio; the 10 highly-significant ratios should include several true enriched samples.

The flavonoid relatives appear to be the most promising agents, as might be expected since the markers are flavonoid factors.

| Agent | Ppm | Ratio | Locus | Dev. | Chrom. |
|---|---|---|---|---|---|
| Acridine orange | 1000 | 0:1 | $\underline{c}_1$ | +* | 9 |
| | 1000 | 10:2 | $\underline{c}_1$ | | 9 |
| | 500 | 11:8 | $\underline{c}_1$ | | 9 |
| Carbohydrate metabolism | | | | | |
| 2, 4-dinitrophenol | 100 | | | | |
| | 50 | | | | |
| Oligomycin | 50 | | | | |
| | 25 | | | | |
| turanose | 5000 | | | | |
| | 2500 | | | | |