4. Chemical analysis of certain mutant aleurone tissue.

The chemical nature of the mutants \underline{c}^{I} , \underline{c}_{1} , \underline{c}_{2} and \underline{r} was studied by using certain diagnostic chemical tests, chromatography and spectroscopy. Chromatograms of all these mutant tissues are colorless and no phenolic substance was detected. Various chemical tests, such as ferric coloration, sodium borohydride-HCl (for detecting flavonone), sodium borohydride-DDQ test (for detecting dihydrochalcones), Pachecko's test (also for detecting flavonone), HCl test (for detecting leucoanthocyanidins), Magnesium-hydrochloric acid test and Zinc-hydrochloric acid test (general), led to the conclusion that none of these mutant tissues accumulates any detectable flavonoid pigments. However, it was found that $\underline{c_1}$ extract (aqueous MeOH) responded positively to acid tests, i.e., effervescence with sodium bicarbonate and reduction of potassium permanganate. On paper chromatography with the solvent mixture ethylacetate: formic acid:water (10:2:3), it gave one colorless spot which fluoresced under UV (long range) with an Rf value of 0.63 and another faint fluorescent spot which runs along with the solvent front. When the chromatograms were sprayed with 1% sodium nitrite in 10% acetic acid and fumed with ammonia, the spot turned yellow. Thus, the preliminary analysis suggests that the accumulated substance in $\underline{\mathbf{c}}_{\mathbf{l}}$ mutant aleurone tissue may be a phenolic acid whose Rf values closely resemble those of chlorogenic acid. Further studies are in progress.

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Soft endosperm genes.

A group of endosperm mutants have been studied which have phenotypes similar to "opaque" but their expression is dependent on duplicate recessive factors. To distinguish them from the floury series (monogenic with dosage effect) and the opaque series (monogenic

recessive), the new genes have been designated <u>soft endosperm</u>. Expression of mutants 5586 and 4918 is dependent on <u>sen</u>, located on chromosome 3, and <u>sen</u> 2, located on chromosome 7. Mutant 4921 is dependent on <u>sen</u> 3, located on chromosome 1, and <u>sen</u> 4, not yet located. The expression of mutant 5595 is dependent on <u>sen</u> 5, located on chromosome 2, and <u>sen</u> 6, located on chromosome 5.

The amino acid profile of these mutants is approximately normal and the amylose-amylopectin ratio is normal.

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1. Partial purification of the catalase-specific inhibitor in maize.

We previously reported evidence of a catalase inhibiting substance active in 24 hr. scutellar extracts that was apparently absent by the fourth day of germination (1). We have since initiated attempts at purification and characterization of the factor.

The inhibitor precipitates in 30-45% saturated ammonium sulfate solutions, as does approximately 80% of the catalase activity. Both activities are also retained by an Amicon XM-100 ultrafilter (100,000 MW exclusion). Gel filtration on Sephadex G-200 yields coincident peaks for inhibitor and catalase activity. In addition, a peak of inhibitor activity is seen in the void volume of the column suggesting an apparent molecular weight of several hundred thousand. This high apparent molecular weight and the copurification suggest the presence of a catalase-inhibitor complex. Attempts to dissociate this complex with urea, heat, high salt, and high and low pH have been unsuccessful. We have recently succeeded in preparing a catalase-sepharose affinity column, and expect it to be an invaluable aid in resolving this problem.