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1. Ribonuclease activity in half opaque-2 kernels.

The defatted mature endosperm powder (200 mg) of normal maize (CM 109), opaque-2 (CM 109), and half opaque ($S_{5}o_{2}$): half normal (S_{5+}) maize was extracted according to the procedure of Wolf (Experimentia 24:890, 1968). Acrylamide (7.5%) gel electrophoresis of the above extract was carried out at 5+ 2°C. The gels were incubated in an RNA solution for one hour. The reaction mixture included yeast RNA in a 0.2 M acetate buffer, pH 5.0. The fixation and staining was done in a 0.2 M acetate buffer containing 1% Lanthan acetate and 1% methylene blue. The stained gels were washed repeatedly with 0.1% acetic acid until the unstained transparent bands of RNase were clearly revealed.

The normal endosperm (CM 109) revealed a single broad but not very clean band, between 1.5 cm and 2.5 cm from the origin. The opaque-2 endosperm on the other hand, revealed four very clear (transparent) and distinct bands occupying a longer area of the gel than the normal counterpart. They extended from 1.5 cm to 3.0 cm from the origin. Among the four bands, the first and fourth were very prominent. The first two bands in the opaque-2 occupy the same position as the single band in the normal.

The normal and opaque tissues of S_5 kernels revealed a still different pattern with an increased number of bands. In S_{5+} three bands were present, the first two being nearer to the origin. The third band occupied the same position as in the normal but was less broad. The opaque tissue of $S_5(S_{5}o_{2})$ revealed five bands, all except the first two being very transparent. The first three bands resemble the pattern of S_{5+}. Bands 4 and 5 of $S_{5}o_{2}$ were not observed in S_{5+}. In general, the pattern of $S_{5}o_{2}$ represented a combination of the patterns of opaque-2 and S_5.

The RNase activity seems to be much higher in opaque than in normal endosperm and its accumulation occurs within 16 days of pollination, the rates being smaller in both endosperms after sixteen days, suggesting

activity of the opaque gene. The presence of more bands and RNase isozymes in S_5o_2 suggests a higher activity compared with S_{5+} or opaque-2; however, the S_{5+} exhibits a higher RNase activity compared to normal or opaque-2, which may be due to genotypic differences.

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2. Luteolinidin in aleurone tissue of the bz_1 mutant.

By using chromatographic (BAW, Forestal), spectrophotometric and chemical techniques, it was found that hydrolysates of methyl alcohol-HCl extracts of bz_1 aleurone contain an orange-red pigment, Luteolinidin (3-deoxycyanidin) and apigeninidin (3-deoxy pelargonidin), in addition to a dark brown pigment. However, apigeninidin was present only in trace amounts. These pigments were absent in the hydrolysates of the single mutants C^I, a_1, r, c_1, c_2, and a_2 and the double mutants C^I bz_1, c_1 bz_1, a_1 bz_1, and a_2bz_1. The a_2 bz_1 hydrolysate yielded cyanidin chloride as a result of conversion of the Leucocyanidin. The double mutant, in bz_1, has shown about a fivefold increase in pigment as determined by a Klett Summersson photoelectric colorimeter.

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3. Chemical nature of an induced salmon silk mutant.

A salmon silk mutant induced by DES in opaque-2 material was subjected to chromatographic, spectrophotometric, and chemical techniques and it was found that the hydrolysates of a methyl alcohol-HCl extract of fresh silks contain an orange-red pigment, Luteolinidin.

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