The recessive mutations for the ten known markers were found to be allelic, with the exception of $\underline{g_1}$ and $\underline{gl_1}$ which need to be tested. The recovered new mutants were found to breed true and allelic studies are in progress.

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4. High protein opaque-shrunken endosperm.

Induced opaque-shrunken endosperm (MNL 44:178) was found to have high protein (18.0%). Preliminary studies suggest that the shrunken-opaque is not allelic to either sh, sh, sh, or bt.

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5. Biochemical nature of bz and bz mutants.

The chemical nature of the accumulated substance in \underline{a}_2 mutant aleurone was reported earlier (MNL 45:169-171). Similar studies were conducted with $\underline{b}\underline{z}_1$ and $\underline{b}\underline{z}_2$ along with certain other double mutant combinations.

The characterization of the isolated substances was made by the following: 1) Rf values; 2) absorption maxima; 3) visible color; 4) color reactions; 5) response to various diagnostic spraying reagents; 6) thin layer chromatography (Silicagel); 7) paper chromatography. Absorption maxima of chromatographically pure compounds were recorded in 5% methanol-hydrochloric-acid solution on UV specord VIS. The relative quantities of the pigments were determined on a Klett-Summerson photoelectric colorimeter.

Genotype	Leucoanthocyanidin	cyanidin	cyanidin-3-glucoside
bz ₁	P	P	A
bz _l pr	P	Ā	A
bz _l in	P	P	A
bz _l a _l	A	A	A
bz ₁ a ₂	P	A	A
b z 2	A	TA	P
bz ₂ in	A	TA	P
bz ₂ a ₁	A	A	A
bz ₂ a ₂	P	A	A

Table 1. Identification of substances accumulated in different genotypes.

(P = Present; A = Absent; TA = Trace amounts)

These studies suggest that $\underline{bz_1}$ and $\underline{bz_2}$ accumulate cyanidin and cyanidin-3-glucoside, respectively, indicating that the $\underline{Bz_1}$ gene may control a glycosidation step. The double mutants, $\underline{a_1}$ $\underline{bz_1}$, $\underline{a_1}$ $\underline{bz_2}$, $\underline{a_2}$ $\underline{bz_1}$, and $\underline{a_2}$ $\underline{bz_2}$, lack these pigments.

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6. The role of the modifying factors, In/in and Pr/pr.

Colorimetric analysis of the pigment levels in $\underline{bz_1}$ in and $\underline{bz_2}$ in suggests that the homozygous recessive in enhances the production of cyanidin in $\underline{bz_1}$ and cyanidin-3-glucoside in $\underline{bz_2}$ tissue. The mutant $\underline{bz_1}$ \underline{Pr} accumulates cyanidin and $\underline{bz_1}$ \underline{pr} pelargonidin. The accumulation of cyanidin in $\underline{bz_1}$ and cyanidin-3-glucoside in $\underline{bz_2}$ indicates that $\underline{Bz_1}$ might be involved in glycosidation. Further the $\underline{Bz_1}$ gene may act prior to $\underline{Bz_2}$ and both of them act after \underline{In} , \underline{Pr} , $\underline{A_1}$ and $\underline{A_2}$. These observations independently confirm the sequence of gene action proposed earlier (MNL 35:95).

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