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# Endosperm Protein Synthesis in Maize Mutants with Increased Lysine Content

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Abstract. *The endosperm proteins of the maize mutants,* opaque-2, opaque-7, floury-2, brittle-2, *and the double mutant of* opaque-2 *and* brittle-2, *were separated into five soluble fractions by the Landry-Moureaux method. As compared to their isogenic normal counterparts, the mutant endosperms had higher concentrations of albumins, globulins, and glutelin-3, and lower concentrations of prolamines. The combination of the* opaque-2 *and* brittle-2 *genes enhanced this difference. Although the four mutant genes are located on three different chromosomes, they exert a similar effect on endosperm protein composition. Five other starch-modifying mutants with high lysine content resemble the* brittle-2 *mutant in endosperm protein composition, when the gene is present either singly or combined with* opaque-2. *Therefore, the pattern of protein synthesis in all maize mutants with high lysine concentrations may be either identical or very similar. Because no synergistic effect on lysine concentration is obtained when* floury-2 *is combined with* opaque-2, *different pathways leading to reduced zein synthesis may exist in the floury and starch-modifying mutants with high lysine concentrations.*

In 1964 Mertz *et al.* (*1*) reported that the *opaque*-2 ($o_2$) gene (chromosome 7) changed the protein composition and increased the lysine content of maize endosperm. Using a copper fractionation method (*2*), they found that the zein concentration of $o_2$ endosperm was lower, and the glutelin concentration was higher, than in normal endosperm. A second maize mutant with high lysine concentrations, *floury*-2 ($fl_2$) (chromosome 4), was identified and reported in 1965 (*3*). Recently, McWhirter (*4*) identified a third maize mutant with high lysine content, designated *opaque*-7 ($o_7$). This appeared as a spontaneous mutation in the inbred line W22; linkage studies (*4*) show it is located on chromosome 10.

In studies of starch modifying mutant genes and their combinations with the $o_2$ gene, we found that the *sugary*-1 ($su_1$), *shrunken*-1 ($sh_1$), *shrunken*-2 ($sh_2$), *shrunken*-4 ($sh_4$), *brittle*-1 ($bt_1$), and *brittle*-2 ($bt_2$) genes increased the lysine content of the endosperm substantially above the isogenic normal control, and each gene had an enhanced effect on lysine when the gene was combined with $o_2$ (*5*). In addition, we found that the distribution of endosperm proteins in these six mutants resembled that found in the three floury maize types with high lysine content ($o_2$, $o_7$, and $fl_2$).

We present data on the $bt_2$ mutant and its combination with $o_2$ as a typical example of the starch-modifying mutants that exhibit more or less comparable degrees of endosperm defectiveness in the mature kernel. We have added, for comparison, the data on $o_2$ and $fl_2$ mutants in the same isogenic background, and have included the $o_7$ mutant, whose complete amino acid and protein patterns have not been reported previously. The $bt_2$ gene is located on chromosome 4 at a locus distinct from that of $fl_2$. The endosperm of the $bt_2$ mutant is translucent and shrunken (*6*).

Near isogenic sublines of $o_2$, $fl_2$, and $bt_2$ of inbred Oh43 were recovered after six backcrosses to the recurrent parent. The double mutant $bt_2o_2$ was isolated by a system of backcrossing and selfing, which permitted the classification for the segregation of the $bt_2$ gene in the background of the $o_2$ gene. The double mutant endosperm is opaque and shrunken, and the mature kernels are comparable in defectiveness to those of the $bt_2$ mutant (*7*).

The dry corn kernels were soaked in distilled water for 30 minutes, and were then separated with a scalpel into pericarp, embryo, and endosperm. The endosperms were dried in air overnight, were defatted with hexane, and were ground to a fine powder in a ball mill. The concentration of nitrogen was determined on the powder by the micro-Kjeldahl technique; 25-mg samples were hydrolyzed with 100 ml of 6*N* HCl under reflux for 24 hours, the acid was removed in a vacuum on a rotary evaporator, and the residue was taken up in *p*H 2.2 citrate buffer. This solution was applied to the columns of an automatic amino acid analyzer (Beckman-Spinco). Data were obtained on single hydrolyzates because duplicate hydrolyzates on many similar maize mutant samples did not differ by more than 5 percent in their lysine content. The concentration of tryptophan was determined in 100-mg samples of defatted powder by a colorimetric method (*8*).

Portions of the powdered defatted endosperms (1 g each) were subjected to fractionation sequence D of the procedure described by Landry and Moureaux (*9*). Fraction I contains proteins soluble in saline (albumins, globulins); fraction II, zein (prolamine); fraction III, glutelin-1; fraction IV, glutelin-2; and fraction V, glutelin-3.

Other data (*5*) show that the embryo

Table 1. Amino acid composition of defatted maize endosperms.

| Amino acid | Amino acid concentration (grams per 100 g of protein) in | | | | | | |
|---|---|---|---|---|---|---|---|
| | Oh43 | | | | | W22 | |
| | + | $fl_2$ | $o_2$ | $bt_2$ | $o_2bt_2$ | + | $o_7$ |
| Lysine | 1.6 | 2.7 | 3.5 | 3.3 | 5.3 | 2.3 | 3.8 |
| Tryptophan | 0.3 | 0.5 | 0.8 | 0.7 | 1.3 | 0.4 | 0.7 |
| Leucine | 16.4 | 15.4 | 12.1 | 12.3 | 8.3 | 15.9 | 12.5 |
| Isoleucine | 4.3 | 4.3 | 4.3 | 4.1 | 4.1 | 4.3 | 4.3 |
| Threonine | 4.0 | 3.9 | 4.4 | 4.4 | 5.5 | 4.0 | 4.4 |
| Methionine | 2.4 | 3.7 | 2.7 | 3.4 | 2.4 | 3.2 | 3.2 |
| Cystine | 2.1 | 1.4 | 2.1 | 2.0 | 2.3 | 2.4 | 1.9 |
| Phenylalanine | 6.8 | 6.1 | 6.0 | 5.6 | 5.2 | 6.7 | 5.2 |
| Tyrosine | 5.9 | 5.1 | 5.2 | 5.1 | 4.9 | 5.8 | 4.9 |
| Valine | 5.2 | 5.2 | 5.9 | 5.6 | 7.0 | 5.7 | 6.7 |
| Histidine | 3.0 | 2.9 | 3.4 | 3.2 | 3.6 | 3.6 | 4.1 |
| Arginine | 3.4 | 5.8 | 5.1 | 5.0 | 7.2 | 3.7 | 5.2 |
| Glycine | 3.3 | 3.2 | 4.9 | 4.6 | 7.4 | 4.0 | 5.2 |
| Alanine | 10.1 | 8.9 | 8.2 | 8.3 | 7.5 | 8.5 | 7.9 |
| Serine | 6.0 | 5.3 | 5.4 | 5.6 | 5.6 | 5.4 | 5.6 |
| Aspartic acid | 7.5 | 7.2 | 9.5 | 8.1 | 10.7 | 6.7 | 9.4 |
| Glutamic acid | 30.0 | 26.4 | 23.6 | 23.6 | 19.0 | 27.4 | 25.5 |
| Proline | 11.3 | 9.5 | 9.8 | 9.6 | 8.9 | 11.5 | 11.0 |
| Protein (percent) | 11.8 | 12.3 | 10.1 | 13.4 | 12.9 | 8.5 | 7.3 |

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Table 2. Nitrogen distribution in maize endosperms.

| Fraction | Soluble nitrogen (percent of total) in | | | | | | |
|---|---|---|---|---|---|---|---|
| | Oh43 | | | | | W22 | |
| | + | $fl_2$ | $o_2$ | $bt_2$ | $o_2bt_2$ | + | $o_7$ |
| I (saline) | 5.8 | 9.2 | 13.6 | 12.1 | 22.3 | 6.9 | 16.6 |
| II (zein) | 59.0 | 49.1 | 26.9 | 26.1 | 2.9 | 40.6 | 20.3 |
| III (glutelin-1) | 5.8 | 9.0 | 8.4 | 15.4 | 5.5 | 15.3 | 12.0 |
| IV (glutelin-2) | 12.7 | 7.6 | 14.0 | 8.7 | 12.2 | 12.8 | 18.8 |
| V (glutelin-3) | 13.8 | 22.0 | 29.2 | 27.9 | 48.0 | 21.0 | 29.5 |
| (Total nitrogen extracted) | 97.1 | 96.9 | 92.1 | 90.2 | 90.9 | 96.6 | 97.2 |

of all four mutants and the double mutant $bt_2o_2$, have similar amino acid patterns, with lysine content ranging from 5.4 to 6.1 percent of total protein.

As compared to their normal isogenic counterparts (Table 1), the four endosperm mutants can be classified as maize mutants with high lysine content. The combination of the $o_2$ and $bt_2$ genes increases the difference in amino acid content: lysine and tryptophan increase to 5.3 and 1.3 percent, respectively, and leucine decreases to 8.3 percent. When whole ground kernels of $o_2$, $bt_2$, and the double mutant were each fed to two inbred Wistar rats at an amount that was 95 percent of the ration, for 10 days, the animals fed the double mutant gained an average of 4.8 g/day, which is 1.6 times that (3.0 g/day) of the animals on the other two diets (*10*). These initial tests suggest that the extra lysine and tryptophan of the double mutant are available for growth purposes. However, because of the shrunken endosperm of the double mutant, one would expect reduced yields for mature grains, making this nutritious kernel economically impractical for animal feeds. Nevertheless, even at twice the current production cost of normal corn (2¢ per pound), the double mutant could have value as a special food for infants, or as an edible corn in the green stage of development.

Table 2 shows the protein distribution in the fractions extracted from each sample. All mutants show a substantial increase in proteins soluble in saline; again, there is an enhanced effect with the double mutant. The zein concentration (fraction II) drops with introduction of any one of the mutant genes; the enhanced effect is pronounced in the double mutant, where zein content drops to 2.9 percent. The major glutelin fraction (glutelin-3, fraction V) increases with the introduction of each one of the mutant genes. The enhanced effect of the $o_2$ and $bt_2$ genes is evident, with this fraction comprising 48 percent of the soluble nitrogen (Table 2, column 6). The endosperm proteins of near isogenic (Oh43), $su_1$, $sh_1$, $sh_2$, $sh_4$, and $bt_1$ also have increases in lysine, albumins, globulins, and glutelin-3, and a reduction in zein, when the gene is present either singly or combined with $o_2$ (*5*).

Complete amino acid patterns (with the exception of tryptophan) have been obtained on the protein fractions listed in Table 2 (*5*). The range of lysine values in these fractions was as follows (grams of lysine per 100 grams of protein): I, 3.7 to 6.3; II, 0.1 to 0.5; III, 0.4 to 0.8; IV, 1.4 to 2.8; and V, 6.4 to 7.1. The increased lysine content (Table 1) for $fl_2$, $bt_2$, $o_2$, $o_2bt_2$, and $o_7$ is due primarily to increases in fractions I and V (proteins high in lysine content), and to decreases in fraction II (proteins low in lysine content) (Table 2).

These studies show that the mutant genes $fl_2$, $o_2$, $bt_2$, and $o_7$ exert a similar effect, namely, a repression of zein synthesis, and a stimulation of albumin, globulin, and glutelin synthesis. Thus, similar effects are observed with mutant genes from three different chromosomes, and a synergistic effect is obtained when $o_2$ (chromosome 7) and $bt_2$ (chromosome 4) are combined to form the double mutant. Since five other starch-modifying mutants high in lysine content resemble the $bt_2$ mutant in protein composition, either singly or when combined with $o_2$, the pattern of protein synthesis in all maize types high in lysine content may be either identical or very similar.

In contrast to the starch-modifying mutant genes, no enhanced effect is observed when $fl_2$ is combined with $o_2$ in the Oh43 background. The double mutant $o_2fl_2$ has a lysine concentration that is intermediate between that of $o_2$ and $fl_2$ (*5*). Therefore, while the pattern of protein synthesis appears to be the same, the pathway leading to reduced zein synthesis in the $fl_2$ mutant may be different from that in the starch-modifying mutants.

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