STARCH SYNTHESIS IN THE MAIZE ENDOSPERM
AS AFFECTED BY STARCH-SYNTHESIZING MUTANTS

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The goal of this project was the elucidation of the pathway of starch biosynthesis in the developing maize endosperm with mutants affecting the process constituting the experimental probes. Studies involving a total of seven different loci were undertaken, with a concentration on four of these:

(1) *brittle endosperm1 (bt1)*. Molecular clones of the locus were obtained via a *dSpm* transposable element tag, and a complete cDNA sequence was obtained. The deduced protein sequence indicated that the BT1 protein was likely an amyloplast membrane metabolite transporter. Further work by Tom Sullivan (funded by USDA-NRICGP) confirmed the amyloplast membrane location of BT1, and work from other labs strongly suggests that BT1 is a transporter for cytoplasmically-synthesized ADP-glucose into the amyloplast where it functions as a substrate for starch synthesis.

(2) *brittle endosperm2 (bt2)*. *Bt2* encodes a subunit of ADP-glucose pyrophosphorylase. An allele called *bt2-8132* was analyzed and shown to have several interesting properties. It has a phenotype, unlike other *bt2* alleles, in being only modestly affected in starch production. The starch produced in mutant *bt2-8132* endosperm has anomalous properties in the Brabender test (performed in collaboration with scientists at American Maize) and could be of potential use in the food industry (a patent has been granted). An apparent indirect effect of the *bt2-8132* mutation is a decrease in the level of an enzyme termed glycosidase-transferase or GLT. This appears to be a bifunctional enzyme that can cleave α -1,6- and α -1,4-glucose bonds, and transfer the released maltose or maltotriose to oligo- or polysaccharide acceptors.

(3) *phosphoglucomutase (pgm)*. Isozymes of PGM, a key enzyme in the interconversion of photosynthate to starch, are found in both cytosol and plastidic compartments. During a screen of mutant effects on enzymes involved in starch synthesis, it was discovered that many maize inbred lines lack the endosperm cytosolic PGM. Though the lack of cytosolic PGM has no discernible phenotypic effects, it is conceivable that the missing PGM is an example of one of several null or hypomorphic alleles that are postulated to be present in any inbred line and that cumulatively reduce vigor and productivity. These cryptic defects would then be repaired when two inbreds, with different combinations of defects, are crossed to produce greater yielding hybrids.

(4) *sugary3 (su3)*. Mutation of *su3* (identified by Phil Stinard) results in kernels with a vitreous appearance like *su1*, but with less wrinkling of the kernel. Mutant *su3* kernels also produce considerable quantities of a water-soluble highly branched polysaccharide, but with structural differences from the phytoglycogen produced in *su1* endosperm. Likewise, mutant *su3* endosperm have altered profiles for debranching enzyme activity, but the profile differs from that of *su1*.

(5) Other loci and projects. *dull1 (du1)* and *sugary2 (su2)* mutations condition decreased total starch and a decrease in the amylopectin fraction. Enzyme studies indicate decreased activity in phosphofructokinase for *su2* and in glucose-6-P kinase for *du1*, but in neither case were these enzyme defects shown to be the primary lesion caused by the mutation.

Genetic and chemical analyses were performed on a line with orange endosperm derived from the *bt2-8132* line (above). Genetic analysis indicates that the orange color may result from the interaction of two loci. Preliminary analysis of carotenoids extracted from orange vs. yellow kernels suggests that the orange color derives from proportionate increases in all of the usual carotenoids.

PUBLICATIONS:

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T. D. Sullivan, L. I. Strelow, C. A. Illingworth & O. E. Nelson, Jr. 1992. Analysis of maize *Brittle-1* alleles and a defective-*Suppressor-mutator*-induced mutable allele. *Plant Cell* 3:1337-1348.

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PATENT:

P90037US: MUTANT MAIZE VARIETY CONTAINING THE *GLT1-1* ALLELE