The amino acid composition of the collective endosperm proteins of opaque-6 mutant is rather similar to that of the opaque-2, opaque-7, and floury-2 mutants. The same amino acids (lysine, arginine, aspartate, glycine) are increased as in those mutants. The decreases observed for proline, alanine, and leucine are also characteristic of these other mutants. It is interesting that in this mutant a familiar pattern of amino acid shifts is associated with a lethal seedling condition.

Yu Ma
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3. Amylo-1,6-glucosidase activity in maize endosperm.

Amylo-1,6-glucosidase (de-branching enzyme) activity was examined in maize endosperms collected 22 days after pollination, frozen on dry ice, and stored at -20°C. The enzymatic activities in the endosperm of several carbohydrate mutants and in the hybrid W64A x W182E (+) endosperms and pollen were determined using a modification of the procedure described by Nelson and Larner (Analyt. Biochem. 33:87-101, 1970). Their method relies on the incorporation of ^{14}C-glucose into glycogen as a means of quantitatively measuring the de-branching activity. ^{14}C-glucose residues that are incorporated into polysaccharide are precipitated by aqueous ethanol on filter paper, while free glucose molecules are soluble in aqueous ethanol.

The pericarp and embryos were removed from endosperms, which were homogenized (in a ratio of 1 g. fresh weight endosperm/1 ml extraction buffer and 1 g. fresh weight pollen/5 ml extraction buffer) in a Virtis blender in chilled 0.01 M citrate buffer (containing 10^{-3}M dithiothreitol) at pH 6.75. This homogenate was filtered through cheesecloth and centrifuged at 31,000 x g for 20 minutes. A 0% to 70% saturation ammonium sulfate cut was taken and, after centrifugation, the precipitated protein was redissolved in extraction buffer. This preparation, after dialysis for about 14 hours against extraction buffer, was used to assay amylo-1,6-glucosidase activity.

The 400 ul incubation mixture contained 100 ul enzyme prep.; 100 ul of D-glucose-^{14}C-UL solution, (15 uCi and 7.5 umoles/ml); and 200 ul

0.10M citrate buffer pH 6.0 containing 4 mg dissolved phytoglycogen. This reaction mixture was incubated at 37°C.

Sixty microliter aliquots were taken from the reaction mixture at time intervals of 0, 10, 35, and 45 minutes after the start of the reaction and added to testtubes containing 0.5 ml 60% ethanol. After centrifugation at 31,000 x g for 20 minutes, the supernatant was decanted, and the precipitated phytoglycogen was rinsed with 95% ethanol and recentrifuged. The ethanol supernatant was decanted, and the phytoglycogen precipitate was dissolved in 0.5 ml 0.2M phosphate buffer, pH7.0. Immediately before filtration on a Millipore filter apparatus, 1.0 ml of 95% ethanol was added and the phytoglycogen was trapped on glass fiber filter paper. The glass fiber filters were dried, added to vials containing PPO-POPOP scintillation fluid, and counted.

There was a lag period of about 30 minutes before appreciable ^{14}C-glucose incorporation was observed. Three 60 ul aliquots were taken 45 minutes after the start of incubation. The incorporation of ^{14}C-glucose was linear with respect to time between 30 and 60 minutes.

In Table 1, amylo-1,6-glucosidase activity is expressed as mumoles glucose incorporated/ 45 minute incubation period/mg. protein and also as mumoles glucose incorporated/45 minute incubation period/endosperm on a 400 ul total incubation volume basis.

Some mutants examined were in heterogeneous genetic backgrounds; this may explain some of the variation in enzymatic activity observed between different mutants.

Amylo-1,6-glucosidase activity was detected in both the pollen and endosperm of the hybrid W64A x W182E. Considerable enzymatic activity was observed in the two _waxy_ alleles examined, _wx-Bl_ and _wx-C_. The absence of amylose in _waxy_ starch does not appear to be due to the lack of a functional de-branching enzyme.

Two mutants, _miniature_ (_mn_) and _de*-92_, had greatly reduced amylo-1,6-glucosidase activities compared to the hybrid W64A x W182E and many of the other mutants examined. The activities of _mn_ and _de*-92_, expressed on a per endosperm basis, were 5.1% and 7.6%, respectively, of the activity of the hybrid W64A x W182E. When enzymatic activities are

Table 1

Amylo-1,6-glucosidase activity in maize endosperm harvested 22 days after pollination and in mature W64A x W182E(+) pollen

Genotype	Year harvested	Soluble protein[1] (mg/ml)	Soluble protein[1] (mg/endosperm)	mumoles glucose incorporated/45 min. incubation period/mg protein (mean + std. error)	mumoles glucose incorporated/45 min. incubation period/endosperm (mean + std. error)
+ pollen	1973	3.12	----	1.97 ± 0.36	----
+ endosperm	1973	3.92	0.87	1.82 ± 0.28	1.57 ± 0.27
wx-C	1973	3.36	0.53	2.11 ± 0.54	1.12 ± 0.30
wx-B1	1973	4.16	0.61	1.43 ± 0.35	0.87 ± 0.21
bt-c	1973	4.64	0.92	1.13 ± 0.14	1.04 ± 0.13
bt2-r	1973	3.52	0.63	3.12 ± 0.37	1.97 ± 0.23
cp	1973	5.28	0.64	1.41 ± 0.25	0.91 ± 0.16
cp2	1973	3.60	0.62	0.59 ± 0.05	0.36 ± 0.03
sh	1971	3.36	0.44	2.70 ± 0.10	1.19 ± 0.05
sh2-c2	1973	3.44	0.80	1.61 ± 0.55	1.28 ± 0.43
sh4-c	1973	1.62	0.46	1.33 ± 0.58	0.61 ± 0.27

Table 1 (Continued)

Genotype	Year harvested	Soluble protein[1] (mg/ml)	Soluble protein[1] (mg/endosperm)	mumoles glucose incorporated/45 min. incubation period/mg protein (mean + std. error)	mumoles glucose incorporated/45 min. incubation period/ endosperm (mean + std. error)
ae-st	1972	3.44	0.47	2.34 ± 0.40	1.10 ± 0.18
du-st	1972	4.24	0.55	2.65 ± 0.64	1.46 ± 0.35
su	1970	6.48	1.09	0.63 ± 0.24	0.68 ± 0.26
W64A su2	1971	4.72	0.53	0.63 ± 0.25	0.33 ± 0.13
mn	1973	6.88	0.31	0.24 ± 0.05	0.08 ± 0.02
de*-Kg	1973	3.12	0.49	0.60 ± 0.25	0.30 ± 0.12
de*-Ke	1972	4.24	0.33	1.93 ± 0.45	0.64 ± 0.15
de*-Kn	1971	4.64	0.73	0.43 ± 0.11	0.31 ± 0.07
de*91	1973	3.68	0.59	1.24 ± 0.42	0.55 ± 0.11
de*92	1971	3.76	0.23	0.51 ± 0.02	0.12 ± 0.01
de*95	1972	4.08	0.56	1.02 ± 0.13	0.57 ± 0.07
de*7005	1972	3.04	0.45	0.78 ± 0.06	0.35 ± 0.03

[1]A 0%-70% saturation ammonium sulfate cut was taken.

expressed on a protein basis, mn and de*-92 activities were 13.2% and 28%, respectively, of the hybrid W64A x W182E activity.

De-branching enzyme activity was qualitatively detected in 3.75% acrylamide-agarose gels which had 0.7% phytoglycogen incorporated into them. A band which stains a deep blue color with I_2-KI was visible in the hybrid W64A x W182E endosperm and pollen and it was detectable in most of the mutants (including wx-C) examined but was absent in mn and de*-92.

Warren Bryce

4. Identical twins of dizygotic origin.

The proportion of twins formed by cleavage polyembryony in plants has been estimated from the excess over one-half of twins concordant in phenotype for a gene heterozygous in one parent. In this form the procedure is a direct extension of the estimation in man of one-egg twins from the excess of like-sexed pairs. Caution concerning applicability of these procedures to twins induced through action of the indeterminate gametophyte (ig) mutation was indicated in an earlier report (Amer. Jour. Botany 58:1-7). Twins were invariably concordant for a gene heterozygous in the ear parent; about one-fourth were discordant when the gene was heterozygous in the pollen parent. The corresponding estimates of one-egg twins are 100% and 50%. The discrepancy was explained by a class of ig female gametophytes that differentiate more than one cell capable of functioning as egg. Two such eggs, of identical genotype, evidently can be fertilized by separate sperm.

Might the excess of twins of concordant phenotype observed when the marker gene was introduced via the pollen parent also originate by some means other than cleavage polyembryony? If the two sperm from one pollen grain were to fertilize two eggs of one embryo sac, the resulting embryos would be fully identical even though of dizygotic origin. One class of twins, comprising about one-fourth of all cases in the previous study, suggested this mode of origin. Although the seedling phenotypes were concordant for the marker gene (R:r), they derived from a kernel of noncorresponding aleurone phenotype. Clearly, sperm from two male gametophytes had participated in double (triple?) fertilization. To test