

groups were significant ( $P < .01$ ), as was the drop in seed set in K10k10 plants as compared to k10k10 plants ( $P < .01$ ).

Data obtained by Weber (MNL 42:56-59) are relevant to these observations. Little if any effect of K10 on the C-Sh-Wx region was found in plants heterozygous for Tp. Although a small increase in recombination may have occurred in female cells, no effect of K10 was found in male flowers.

The results indicate that the Tp9 region (when homozygous) is sensitive to the presence of both B chromosomes and K10. Increased recombination has been demonstrated, however, only when crossing over is determined through the female. Furthermore, the data from this sample suggest that K10 has an adverse effect on the reproductive capacity of the organism.

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Edward J. Ward

UNIVERSITY OF WISCONSIN  
Madison, Wisconsin

1. The mutations, de\*-91 and de\*-92.

Two recessive, defective seed mutants, de\*-91 and de\*-92 were sent to this laboratory by Francesco Salamini (Montanaso). Both are small-seeded papery pericarp mutants that are distinguishable from normal kernels on the same ear three weeks after pollination. They are not allelic in spite of their phenotypic similarity. Plants grown from mutant seeds are normal.

Neither mutant is allelic to bt, bt2, sh, sh2, sh4, mn, or o5.

In extracting seeds harvested 22 days post-pollination in order to test the activity of enzymes involved in starch synthesis, it was observed that the supernatant fraction following homogenization with an equal weight of buffer and centrifugation lacked the pronounced opalescence of such extracts from other mutants or from normal seeds. Assays of the protein content of such supernatants showed that the soluble protein content of de\*-91 and de\*-92 is abnormally low (Table 1).

Table 1

The soluble protein content\* of mutant and non-mutant endosperms  
22 days after pollination

Genotype	Mg Protein/g Fresh Wt.	Mg Protein/Endosperm
W64A	8.3	1.3
W64A o2	8.5	1.3
W64A X 182E	6.7	2.2
de*-91	3.5	0.6
de*-92	4.0	0.6
de*-95	7.8	1.5
de*-Ke	8.4	1.3
de*-Kg	8.0	1.5
de*-Kn	8.4	1.9
de*-7005	7.2	2.0

\*Protein content measured by the Lowry method with BSA as a standard.

In view of the low content of soluble protein, we extended the observations to the storage proteins of the mature endosperm to ascertain if there is a coordinate reduction in the storage proteins. Accordingly, 4 g of dry, defatted powder prepared from mature endosperms of de\*-91, de\*-92, and 6004-3 (normal seeds from an ear segregating an opaque mutant) were extracted according to the method of Landry and Moureaux (Bull. Soc. Chim. Biol. 52:1021-1037, 1970). The results are given in Table 2. It should be noticed that many more endosperms of the mutant stocks comprise the 4 g of material analyzed. The protein content per endosperm for the mutant endosperms as compared to normal is similar for total protein and for each solubility fraction. Therefore, the constraints on protein synthesis apply to all the proteins being synthesized. The higher protein content of the endosperm powders from the mutants as compared to normal is interpreted as indicating that the constraints on protein synthesis noted here result in even more severe restrictions on starch synthesis.

Table 2

Protein fractions from mature endosperms of 6004-3 (2),  
de\*-91, and de\*92

Solvent		6004-3	de*-91	de*92
Na Cl 0.5M	Mg. Protein*	40.5	50.3	46.0
	Mg. Protein/Endo.	3.1	1.1	1.0
	% Protein	12.0	10.6	9.0
Isopropanol 55%	Mg. Protein	134.5	192.1	236.9
	Mg. Protein/Endo.	10.4	4.4	5.3
	% Protein	40.0	40.5	46.5
Isopropanol plus 2-mercaptoethanol	Mg. Protein	21.1	25.4	32.3
	Mg. Protein/Endo.	1.6	0.6	0.7
	% Protein	6.3	5.4	6.3
Borate Buffer pH 10 plus 2-mercaptoethanol	Mg. Protein	30.2	19.9	23.7
	Mg. Protein/Endo.	2.3	0.5	0.5
	% Protein	8.6	4.2	4.7
SLS** 0.5 plus 2-mercaptoethanol	Mg. Protein	72.2	97.7	85.9
	Mg. Protein/Endo.	5.6	2.2	1.9
	% Protein	21.4	20.6	16.9
Unextracted Protein	Mg. Protein	37.6	88.4	84.7
	Mg. Protein/Endo.	2.9	2.0	1.9
	% Protein	11.1	18.7	16.6
Mg. Protein (Kjeldahl)		376.0	580.0	625.6
Mg. Protein Recovered		337.4	473.8	509.5
Total Protein Mg./Endo.		29.1	13.2	13.9
No. Endosperms/4g		12.9	44.0	44.9
% Protein (Kjeldahl)		9.4	14.5	15.7

\*Crude protein including free amino acids

\*\*Sodium lauryl sulfate

When tested for activity of various enzymes concerned with starch synthesis, the mutant extracts have generally had normal levels of activity when stated on the basis of protein content. The exceptions to this observation have been the starch granule-bound nucleoside diphosphate glucose-starch glucosyl transferase of de\*92 which is very low relative

to normal or any mutant except waxy and the amylo-1,6-glucosidase of de\*-92. At the same time, the amylose content of the starch produced by de\*-92 is only 12% as compared to 25% in normal and 0% in waxy. It is not established, however, that the low glucosyl transferase activity and the resultant low amylose content of the de\*-92 stock investigated is conditioned by the de\*-92 mutation. It could have its basis in an intermediate waxy allele present in the de\*-92 line. See the accompanying report by Warren Bryce for more detailed information on amylo-1,6-glucosidase activity in endosperms of de\*-92.

It is not concluded that the low protein content of these mutants indicates inefficiency at a step in transcription or protein synthesis. The shrunk-4 mutant, which also has a low protein content at all stages of development of the seed, appears to be partially blocked in pyridoxal phosphate synthesis as the primary mutational lesion. The lower protein content is a secondary consequence of the lessened availability of pyridoxal phosphate. There are obviously other possible defects in reactions not directly concerned with protein synthesis that could result in lowered rates of protein synthesis.

Don Foard\*  
Yu Ma  
Oliver Nelson

\*Biology Division, Oak Ridge National Laboratory, operated by Union Carbide Corporation for the Atomic Energy Commission.

## 2. The endosperm proteins of opaque-6.

We reported no change in the amino acid profile of the collective endosperm proteins of opaque-6 (MNL 46:203). A detailed re-examination of the endosperm proteins of the homozygous opaque-6 and normal seeds from the same ear has been done by the modified Osborn-Mendel procedure. Since the results are not in accord with our previous report, we take this opportunity to offer a correction. The results of protein fractionation and amino acid analysis are given in Tables 1 and 2, respectively.

As shown in Table 1, the major differences between the mutant and its normal counterpart occurred in water-soluble and 70 percent ethanol-soluble fractions. The amount of zein (70 percent ethanol-soluble proteins) in normal endosperms was 9.6 mg per endosperm, which constituted 62.6