STUDIES ON CORN PROTEINS. VI. ENDOSPERM PROTEIN CHANGES IN SINGLE AND DOUBLE ENDOSPERM MUTANTS OF MAIZE[1]

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ABSTRACT

The endosperm proteins of the maize mutants, *floury*-2, *opaque*-2, *opaque*-7, *sugary*-1, *shrunken*-1, *shrunken*-2, *shrunken*-4 and *brittle*-1, and double-mutant combinations of these with *opaque*-2, were separated into five soluble fractions by the Landry-Moureaux method. As compared to their isogenic normal counterparts, all single mutants had higher concentrations of albumin, globulin, and glutelin, and lower concentrations of prolamine. The combination of *opaque*-2 with *floury*-2 or *opaque*-7 did not increase lysine above that in the single mutants. The combination of *opaque*-2 with any of the other five mutants increased levels of albumin, globulin, and glutelin above those found in the single mutants. The double mutants showed an almost complete suppression of prolamine synthesis and the lysine levels were higher than in the single mutants.

In 1972, Misra and co-workers (1) reported that the combination of the *brittle*-2 and *opaque*-2 genes drastically altered the protein distribution in the endosperm. In contrast to the normal counterpart, the double-mutant endosperm contained high levels of albumins, globulins, and true glutelins, and less than 3% zein. We have extended these studies to include seven other endosperm mutants.

MATERIALS AND METHODS

Near isogenic sublines of *floury*-2 (fl_2), *sugary*-1 (su_1), *shrunken*-1 (sh_1), *shrunken*-2 (sh_2), *shrunken*-4 (sh_4), and *brittle*-1 (bt_1) in addition to *opaque*-2 (o_2) of inbred Oh43, were recovered after six backcrosses to the recurrent parent. The double-mutant combinations fl_2o_2, su_1o_2, sh_1o_2, sh_2o_2, sh_4o_2, and bt_1o_2 were

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isolated by a system of backcrossing and selfing, which permitted the classification for the segregation of each of the six single-endosperm mutant genes in the background of the *opaque*-2 (o_2) gene (2). On drying, the double-mutant endosperms of all, except the fl_2o_2 are collapsed, and in the case of su_1o_2 wrinkled, and the mature kernels are comparable in defectiveness to those of the corresponding single-endosperm mutants (1,2). The *opaque*-7 (o_7) mutant and its isogenic normal counterpart, inbred W22, were obtained from K. S. McWhirter (3), who also supplied the double-mutant o_2o_7, and F_3 segregate from the cross W23XL317 o_7.

TABLE I
Landry-Moureaux Fractionation Sequence D

Fraction	Solvent[a]		Time of Agitation min.	Protein Fractions
I	NaCl 0.5 M	(4°C.)	60	Albumins
			30	Globulins
			30	
			15	
	Water		15	
II	Isopropanol 70% (v./v.)	(20°C.)	30	
			30	Zein
			30	
III	Isopropanol 70% (v./v.)		30	
	+ 2-ME 0.6% (v./v.)	(20°C.)	30	Zein-like
IV	Borate buffer pH 10		60	
	+ 2-ME 0.6% (v./v.)	(20°C.)	30	Glutelin-like
V	Borate buffer pH 10		60	
	+ 2 ME 0.6% (v./v.)		30	Glutelin
	+ SDS 0.5% (w./v.)	(20°C.)	15	Residue

[a]2-ME: 2-mercaptoethanol, borate buffer:borate, NaOH, NaCl μ 0.5; SDS:sodium dodecyl sulfate.

TABLE II
Nitrogen Distribution in Maize Endosperms[a]

Fraction	Inbred Genotype	Oh43				W22		
		+	o_2	fl_2	fl_2o_2	+	o_7	o_7o_2
I (saline)		5.8	13.6	9.2	17.0	6.9	16.6	17.6
II (zein)		59.0	26.9	49.1	25.0	40.6	20.3	8.7
III (zein-like)		5.8	8.4	9.0	15.2	15.3	12.0	15.1
IV (glutelin-like)		12.7	14.0	7.6	9.9	12.8	18.8	21.3
V (glutelin)		13.8	29.2	22.0	24.8	21.0	29.5	33.3
(Total N extracted)		97.1	92.1	96.9	91.9	96.6	97.2	96.0

[a]Per cent of soluble nitrogen.

The methods used for separation of endosperms and the determination of total nitrogen and amino acids were described previously (1). The endosperm was finely ground and defatted. The fractionation of proteins followed the Landry-Moureaux method (4) outlined in Table I. To obtain the first fraction, ten parts by weight, of 0.5M sodium chloride solution was added to the endosperm powder (usually 1 g) and the mixture was stirred for 60 min. at 4°C. The mixture was then centrifuged and the extract saved. The residue was treated again with the same volume of saline and stirred for another 30 min. The extraction was repeated a third time for 30 min., and finally the residue was extracted with the same volume of water for 15 min. This was repeated once again for 15 min., and the five extracts combined to give fraction I. The residue was then treated with 10 volumes of 70% isopropanol at 20°C. for three 30-min. periods as outlined in Table I to give fraction II, the residue then reserved for isolation of fraction III, etc.

The first fraction contained the albumins and globulins, the free amino acids and small peptide fragments, and any other saline-soluble compounds. Fraction II contained the prolamine, zein, and fraction III contained the zein-like proteins that were soluble in alcohol after the disulfide bonds in the protein had been reduced with 2-mercaptoethanol. The fourth fraction contained proteins that have some characteristics of glutelin, and the fifth fraction contained the true glutelin, which is a complex high-molecular-weight mixture of proteins that could be solubilized only by treatment with a reducing agent and a detergent (sodium dodecyl sulfate), at alkaline pH.

The nitrogen content of each of these five fractions was determined by micro-Kjeldahl, and the residue left after extraction was also analyzed for nitrogen.

TABLE III
Amino Acid Composition of Defatted Maize Endosperms[a]

Inbred	Oh43						W22		
Genotype	+	*o*$_2$	*fl*$_2$	*fl*$_2$*o*$_2$	*bt*$_2$	*bt*$_2$*o*$_2$	+	*o*$_7$	*o*$_7$*o*$_2$
Lysine	1.6	3.5	2.7	2.7	3.3	5.3	2.3	3.8	3.5
Tryptophan	0.3	0.8	0.5	0.8	0.7	1.3	0.4	0.7	0.7
Leucine	16.4	12.1	15.4	12.5	12.3	8.3	15.9	12.5	10.7
Isoleucine	4.3	4.3	4.3	4.1	4.1	4.1	4.3	4.3	4.1
Threonine	4.0	4.4	3.9	3.7	4.4	5.5	4.0	4.4	3.4
Methionine	2.4	2.7	3.7	2.2	3.4	2.4	3.2	3.2	2.2
Cystine	2.1	2.1	1.4	2.2	2.0	2.3	2.4	1.9	2.5
Phenylalanine	6.8	6.0	6.1	5.5	5.6	5.2	6.7	5.2	4.0
Tyrosine	5.9	5.2	5.1	5.0	5.1	4.9	5.8	4.9	3.9
Valine	5.2	5.9	5.2	5.1	5.6	7.0	5.7	6.7	5.4
Histidine	3.0	3.4	2.9	2.8	3.2	3.6	3.6	4.1	4.1
Arginine	3.4	5.1	5.8	4.3	5.0	7.2	3.7	5.2	4.8
Glycine	3.3	4.9	3.2	3.9	4.6	7.4	4.0	5.2	4.3
Alanine	10.1	8.2	8.9	7.6	8.3	7.5	8.5	7.9	5.5
Serine	6.0	5.4	5.3	4.6	5.6	5.6	5.4	5.6	3.9
Aspartic acid	7.5	9.5	7.2	8.7	8.1	10.7	6.7	9.4	7.1
Glutamic acid	30.0	23.6	26.4	24.9	23.6	19.0	27.4	25.5	18.5
Proline	11.3	9.8	9.5	9.4	9.6	8.9	11.5	11.0	10.0
% Protein	11.8	10.1	12.3	10.4	13.4	12.9	8.5	7.3	7.6

[a]Amino acid levels as per cent of total protein (N × 6.25).

RESULTS AND DISCUSSION

Nitrogen distribution in the Oh43 series. Table II shows the distribution of protein nitrogen in the endosperm of the double mutant *fl*$_2$*o*$_2$. The data on the normal counterpart and single mutants are from the literature (1). In the normal Oh43, the saline fraction makes up only 5.8% of the total protein, whereas the zein fraction accounts for 59% of the total protein extracted. Protein in the zein-like fraction was small (5.8%), the glutelin-like fraction intermediate (12.7%), and the true-glutelin fraction only slightly higher (13.8%). The extraction of protein nitrogen in normal Oh43 was excellent with only 3% remaining in the residue. The *o*$_2$ mutant of Oh43 showed a large increase in the saline and glutelin-protein fractions and a decrease in zein as compared to the normal inbred (Table II). In contrast, the introduction of the *fl*$_2$ gene caused a moderate rise in the level

TABLE IV
Nitrogen Distribution in *Opaque*-2 and Several Nonfloury Endosperm Mutants[a]

Fraction	Endosperm Genotype (Oh43)							
	+	*o*$_2$	*su*$_1$	*sh*$_1$	*sh*$_2$	*bt*$_1$	*bt*$_2$	
I	5.8	13.6	11.9	8.2	12.3	25.7	8.8	12.1
II	59.0	26.9	27.1	43.7	29.4	30.8	36.0	26.1
III	5.8	8.4	21.9	12.3	9.4	7.7	16.4	15.4
IV	12.7	14.0	9.1	14.4	15.0	8.3	8.3	8.7
V	13.8	29.2	22.8	16.3	23.6	23.6	27.4	27.9
Total N extracted	97.1	92.1	92.8	94.9	89.7	96.1	96.9	90.2
Lysine (% of total protein)	1.6	3.5	1.8	1.9	2.7	3.0	2.3	3.3
Tryptophan (% of total protein)	0.3	0.8	0.3	0.6	0.7	0.8	0.5	0.7

[a]Per cent of total nitrogen in endosperm.

TABLE V
Nitrogen Distribution in Endosperms of Double-Combination Mutants of Maize[a]

Fraction	*su*$_1$*o*$_2$	*sh*$_1$*o*$_2$	*sh*$_2$*o*$_2$	*sh*$_4$*o*$_2$	*bt*$_1$*o*$_2$	*bt*$_2$*o*$_2$
I	22.7	39.9	25.3	43.3	23.3	22.3
II	3.0	1.8	1.2	6.5	2.7	2.9
III	9.0	1.6	1.1	3.9	2.5	5.5
IV	14.2	16.4	26.1	8.7	13.1	12.2
V	45.3	32.2	35.4	26.8	50.2	48.0
Total N extracted	94.2	91.9	89.1	89.2	91.8	90.9
Lysine (% of total protein)	3.9	4.8	4.2	4.0	4.8	5.3
Tryptophan (% of total protein)	0.8	1.2	1.2	1.2	1.4	1.3

[a]Per cent of total nitrogen.

of saline-soluble protein, a modest drop in the level of zein, a reduction in the glutelin-like fraction, and an increase in the glutelin fraction that was intermediate between normal and o_2. The lysine level in fl_2o_2 resembles that in fl_2 and is lower than that in o_2 (Table III).

In the combination of fl_2 with o_2 there are no major changes in any of the fractions above those observed for o_2. In contrast to fl_2o_2, with the double-mutant bt_2o_2, there is an increase in saline-soluble fraction, almost complete elimination of zein, and an increase in glutelin (see Table V).

Nitrogen distribution in the W22 series. The data in Table II show that the o_7 mutant in the W22 background brings about changes that resemble those observed with o_2 in Oh43. Genetic differences and/or the lower protein level in normal W22 (Table III) compared with normal Oh43 may be responsible for its lower zein content (40.6%). The combination of the o_7 and o_2 genes gives a marked reduction in zein from 20 to 8.7%, with little change in the other fractions. These changes do not lead to an elevated level of lysine above that of o_2 alone in the Oh43 background as shown in Table III. In contrast, the double-mutant combination Oh43 bt_2o_2 gives levels of lysine which are higher than that of the respective single mutants (Table III).

Nitrogen distribution in nonfloury endosperm mutants. Presented in Table IV are the endosperm nitrogen distributions observed in the six near-isogenic non-floury endosperm mutants. The saline fraction is variable but higher in all instances than in the normal counterpart (8.2 to 25.7% compared with 5.8%). Zein is lower in all the single mutants than in the normal counterpart (26.1 to 43.7% compared with 59.0%). Zein-like fraction is higher in the single mutants (7.7 to 21.9% compared with 5.8%), and the glutelin-like fraction ranges from 8.3 to 15% compared with 12.7% in the normal. Finally, the glutelin fraction in the single mutants is higher than in the normal counterpart (16.3 to 27.9% compared with 13.8%). The lysine content of the single mutants is variable, but all values are higher than that observed in the normal counterpart (1.8 to 3.3% compared with 1.6%). Except in one case (su_1), the tryptophan levels are higher in the single mutants than in the normal counterpart.

Table V shows that the double-mutant combinations of the five endosperm mutants with o_2 give the same enhancing effect observed previously (1) with the bt_2o_2 combination. The saline fraction ranges from 22.3 to 43.3% compared with 5.8% in normal; the zein fraction ranges from 1.2 to 6.5% compared with 59.0% in normal; the zein-like fraction ranges from 1.1 to 9.0% compared with 5.8% in normal; the glutelin-like fraction ranges from 8.7 to 26.1% compared with 12.7% in normal; and the glutelin fraction shows a higher level than that found in the normal counterpart (26.8 to 50.2% compared with 13.8%). These changes are reflected in the lysine values which varied from 3.9 to 5.3% in the double mutants compared with 1.6% in the normal, and 0.8 to 1.4% tryptophan as compared with 0.3% in the normal.

It may be concluded from these data that, with the possible exception of sh_4o_2, there is almost a complete suppression of zein synthesis in the double-mutant combinations. It is obvious from the levels of lysine and tryptophan in these six double-mutant endosperms that the whole kernels will have a high nutritional value.

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