STUDIES ON CORN PROTEINS. VII. DEVELOPMENTAL CHANGES IN ENDOSPERM PROTEINS OF HIGH-LYSINE MUTANTS[1]

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ABSTRACT

The endosperm proteins of the near-isogenic maize mutants *opaque*-2, *brittle*-2, the double mutant of *opaque*-2 and *brittle*-2, and the normal counterpart were separated into fractions by the Landry-Moureaux method. As compared to the normal counterpart, all three mutants had higher concentrations of albumins and globulins throughout seed development. The highest concentrations of albumins and globulins were observed 14 days post-pollination, with a steady decrease 21, 28, 35, 42, and 49 days post-pollination. In the normal counterpart, zein production was evident 14 days post-pollination and reached a peak 42 days post-pollination. The zein level attained in the normal endosperm in 14 days was not reached until the 18th and 21st day, respectively, in *brittle*-2 and *opaque*-2. This delay in onset of zein synthesis, along with slower rates of synthesis, reduced total production of zein to less than 50% of the normal counterpart. In the double mutant of *opaque*-2 and *brittle*-2, zein formation was not apparent at any time during development.

Previous reports (1,2,3,4) with mature seeds of corn (*Zea mays* L.) show that the lysine level of the endosperm can be increased by introduction of any one of the nine specific mutations: *opaque*-2 (o_2), *floury*-2 (fl_2), *opaque*-7 (o_7), *brittle*-1 (bt_1), *brittle*-2 (bt_2), *sugary*-1 (su_1), *shrunken*-1 (sh_1), *shrunken*-2 (sh_2), and *shrunken*-4 (sh_4). These mutations are located on six different chromosomes. Mutations which increase the lysine level of barley (5,6,7) and sorghum (8)

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endosperm have also been identified. Double mutants of maize containing the o_2 gene, in combination with each of the other eight mutant genes which raise endosperm lysine level, have also been studied (2,3,4). Combination of the o_2 gene with either one of the two floury genes, fl_2 or o_7, gives lysine levels resembling those of the single genes fl_2 and o_7. Combination of the o_2 gene with any one of the six nonfloury genes bt_1, bt_2, su_1, sh_1, sh_2, or sh_4 gives lysine levels higher than those of the single genes with complete or almost complete suppression of zein synthesis.

The data presented below were obtained on representative developing mutants and complement the data previously reported (1,2) on mature seeds. For these studies, the normal counterpart and near-isogenic versions of the o_2, bt_2, and o_2bt_2 mutants were chosen.

MATERIALS AND METHODS

Near-isogenic sublines of o_2 and bt_2 of the maize inbred Oh43 were recovered after six backcrosses to the recurrent parent. The double mutant combination bt_2o_2 was isolated by a system of backcrossing and selfing, which permitted the classification for the segregation of the bt_2 endosperm mutant gene in the background of the o_2 gene (4). The corn samples were grown at the Purdue Agronomy Farm in 1972. The ears were collected 14, 21, 28, 35, 42, and 49 days after pollination and frozen immediately in Dry Ice. Three replications of each sample were collected and stored in a freezer.

Kernels were cut from the ears, the endosperm of each sample was dissected out in the frozen state, and lyophilized endosperms from the three replications for each day of collection were mixed to give one composite sample. The composite samples were then crushed and powdered in a ball-mill, defatted, and after drying overnight were used in fraction studies.

Fractionation of endosperm proteins was carried out using the Landry and Moureaux method (3). The five fractions obtained are designated as I (albumin, globulin, free amino acids); II (zein); III (zein-like); IV (glutelin-like); and V (true glutelin). The nitrogen content of the endosperm samples and various fractions was determined by the micro-Kjeldahl method. Lysine was determined in the various fractions of mature seeds as described previously (2). Protein values were calculated by multiplying nitrogen by 6.25.

RESULTS AND DISCUSSION

Total Protein Levels

All endosperm samples contained relatively high levels of protein 14 days post-pollination (Table I). There was a marked reduction during the next 7 days due to an increased rate of carbohydrate synthesis. From the 21st to the 49th days, the level of protein remained essentially constant in the normal and o_2 endosperms. In the bt_2 endosperm, the protein level was unusually high (22%) 14 days post-pollination and remained higher than that found in the normal or o_2 endosperms because of the defect in starch biosynthesis caused by this mutant gene (4). The double mutant of o_2 and bt_2 had elevated protein levels which usually exceeded those found in the bt_2, suggesting an even greater reduction in starch biosynthesis.

Nitrogen Distribution

Fourteen Days Post-Pollination. The albumin-globulin fraction (fraction I) predominated, ranging from 57% of the total nitrogen in the normal to 83% in the double mutant (Table II). Zein synthesis (fraction II) was measurable in the normal counterpart endosperm (9%) but barely detectable in the mutants (0.5 to 1.3%). The zein-like fraction (fraction III) was four times higher in the normal than in the mutants. The glutelin-like fraction (fraction IV) was consistently low and about 3% of the total. The glutelin fraction (fraction V) was higher in the normal and o_2 than in the bt_2 and double mutant. The rate of synthesis of the glutelin fraction was unusually low in the double mutant, bt_2o_2, constituting only 4.4% of the nitrogen. This was due to the unusually high level of fraction I biosynthesis.

Twenty-One Days Post-Pollination. Fraction I decreased as per cent of the total nitrogen in all endosperms, with a 30% decrease in the normal, o_2, and bt_2, and a smaller decrease (12%) in the double mutant. The proportion of true zein increased more than threefold in the normal, about tenfold in o_2 and bt_2, and showed no change in the double mutant. Fractions III and IV increased in all endosperms with 21-day values in a narrow range of 5.7 to 7.8%, except for the double mutant where fraction III remained low (1.7%). Fraction V showed little change in the normal but increased in the mutants.

Twenty-Eight Days Post-Pollination. Fraction I showed moderate decreases in the normal and single mutants and a much larger decrease in the double mutant. The proportion of true zein increased again in the normal and o_2 but remained the same in the bt_2 and double mutant. Fractions III and IV increased moderately in all endosperms, and fraction V showed very little change, except for the double mutant, where it increased nearly threefold. There was a compensatory drop in fraction I of the double mutant.

Thirty-Five Days Post-Pollination. Fraction I dropped slightly in all endosperms, whereas fraction II showed an increase of 7.5 percentage points in the normal, only a minor increase in the single mutants, and none in the double mutant. Fractions III and IV did not change significantly except for the double mutant, where fraction IV increased several per cent. Fraction V was reduced by 6% in the normal, remained about constant in the single mutants, and increased from 20 to 27% in the double mutant.

Forty-Two Days Post-Pollination. Fraction I dropped in all endosperms. Fraction II increased in the normal and bt_2 but remained constant in o_2 and the

TABLE I
Percentage Protein in Defatted Endosperms

Days[a]	Normal Counterpart	o_2	bt_2	bt_2o_2
14	18.7	16.9	22.1	18.8
21	13.7	11.8	16.6	15.3
28	11.9	10.4	13.7	16.6
35	12.3	10.4	14.7	18.2
42	12.4	10.1	15.0	17.7
49	12.2	10.4	16.3	18.3
[b]	11.8	10.1	13.4	12.9

[a]After pollination.
[b]Seeds harvested in 1970 at maturity (4).

TABLE II
Nitrogen Distribution in Developing Endosperms

Days[a]	Fraction[b]	+		o_2		bt_2		bt_2o_2	
14	I	57.2		69.5		80.9		83.4	
	II	9.1		1.0		1.3		0.5	
	III	4.5		1.0		0.9		0.7	
	IV	3.7		3.2		2.5		3.1	
	V	21.4		23.5		10.9		4.4	
	Total	95.9		98.2		96.5		92.1	
21	I	27.9		39.0		46.0		71.5	
	II	32.6		10.1		15.5		0.7	
	III	7.0		5.7		7.1		1.7	
	IV	6.7		7.8		7.8		6.8	
	V	22.5		37.4		21.1		6.9	
	Total	96.7		100.0		97.5		87.6	
28	I	20.2		32.5		42.0		55.0	
	II	41.5		17.9		16.0		0.6	
	III	8.0		7.4		9.7		2.5	
	IV	8.1		8.8		10.0		10.4	
	V	21.1		33.1		20.7		20.5	
	Total	98.9		99.7		98.4		89.0	
35	I	16.3		27.6		35.6		46.7	
	II	48.9		19.1		19.7		0.6	
	III	8.6		8.9		8.6		2.2	
	IV	8.1		9.8		11.1		14.2	
	V	15.0		31.6		19.8		27.0	
	Total	96.9		97.0		94.8		90.7	
42	I	13.9		24.1		27.0		35.3	
	II	55.4		19.2		23.9		0.6	
	III	7.0		12.2		9.1		0.7	
	IV	8.3		10.7		13.8		17.7	
	V	13.1		31.1		22.4		33.4	
	Total	97.7		97.3		96.2		87.7	
49	I	13.0		19.8		23.2		29.0	
	II	50.9		22.4		24.8		0.7	
	III	5.7		12.8		10.0		0.7	
	IV	8.7		9.7		13.3		18.5	
	V	13.1		31.2		25.3		39.0	
	Total	91.4		96.0		96.6		87.9	
Mature (1970 harvest)	I	5.8	(6.3)[c]	13.6	(5.8)	12.1	(4.3)	22.3	(3.7)
	II	59.0	(0.2)	26.9	(0.2)	26.1	(0.1)	2.9	(0.6)
	III	5.8	(0.6)	8.4	(0.5)	15.4	(0.4)	5.5	(0.8)
	IV	12.7	(1.8)	14.0	(2.9)	8.7	(1.7)	12.2	(1.7)
	V	13.8	(6.4)	29.2	(6.7)	27.9	(7.0)	48.0	(6.9)
	Total	97.1		92.1		90.2		90.9	

[a]After pollination, + = normal counterpart.
[b]Per cent of total nitrogen in endosperms.
[c]g lysine per 100 g protein (9).

double mutant. Fraction III showed no consistent trend, changing only slightly in the normal and bt_2, increasing in o_2, and decreasing in the double mutant. Fractions IV and V remained fairly constant in all endosperms with moderate increases in both fractions in bt_2 and the double mutant.

Forty-Nine Days Post-Pollination. Fraction I remained constant in the normal but dropped in the three mutant endosperms. Fractions II, III, and IV remained constant in all endosperms, and fraction V remained constant in the normal and o_2 and increased in the bt_2 and the double mutant.

Mature Seeds

The data on mature seeds of the same genotypes from an earlier harvest (1970) are presented in Table II for comparison. They resemble the values obtained at 49 days post-pollination in the 1972 harvest. Data on amino acid composition of the fractions from mature seeds of the 1970 harvest have been published previously (9) and are listed in parentheses for mature seeds in Table II. It can be seen that fractions I and V contain high levels of lysine (3.7–7%) in not only mutant endosperms but in the normal counterpart as well. Fractions II and III are low in lysine (0.1–0.8%) and fraction IV is intermediate (1.7–2.9%) in both normal and mutant genotypes. Thus, it is the *shift* in levels of fractions in the endosperm, not the change in lysine content of a fraction, that determines the mean lysine level which is attained in the mature endosperm.

SUMMARY

The level of fraction I is consistently lower in the normal throughout development and is consistently at the highest level in the double mutant.

In the normal counterpart, zein production was in progress 14 days post-pollination, rising steadily to a peak 42 days post-pollination. The zein level attained in the normal in 14 days was not reached until approximately the 18th and 21st day, respectively, in bt_2 and o_2. This delay in onset of zein synthesis, together with slower rates of synthesis, reduced total production of zein to 50% of the normal counterpart on the 49th day. In the double mutant, zein formation was not apparent at any time during development.

Fraction III never accounted for more than about 17% of the total nitrogen in any of the endosperms, showed slight increases in bt_2 and o_2 during development, and was consistently low in the double mutant.

Fraction IV followed similar small increases in all endosperms 14 to 28 days post-pollination, leveled off in the normal and o_2, and increased in bt_2 and the double mutant.

Fraction V (true glutelin) did not show consistent trends. The normal started at a moderately high level (20%) and dropped to a low level at 49 days. The o_2 and bt_2 had increases in fraction V between the 14th and 21st day and then leveled off. Only the double mutant showed constant increases in this fraction throughout development of the endosperm.

Since the albumins and globulins (fraction I) and the true glutelins (fraction V) contain high levels of lysine and zein (fraction II) contains a low level, the higher levels of lysine observed in o_2, bt_2 and the double mutant of o_2 and bt_2 can be explained on the basis of a less-than-normal decrease in fraction I, significant increases in fraction V, and less than normal or no increase in fraction II during

seed development. In contrast, in normal endosperms, fractions I and V decrease and fraction II increases substantially during seed development, thus depressing lysine levels in the mature seed.

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