

# STUDIES ON CORN PROTEINS. VIII. FREE AMINO ACID CONTENT OF *OPAQUE-2* DOUBLE MUTANTS<sup>1</sup>

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## ABSTRACT

The ninhydrin method was used to determine the free amino acid content of 14 maize endosperm mutants and their double-mutant combinations with *opaque-2* in the Oh43, W64A, C103, and B37 inbred lines, and hybrid combinations of these lines. In all combinations studied, the introduction of the *opaque-2* gene invariably raised the free amino acid content of the kernel above that found in the single mutant, inbred, or hybrid line.

The endosperm of *opaque-2* (*o<sub>2</sub>*) maize has a higher level of free amino acids than the normal counterpart. Mertz *et al.* (1) developed a rapid ninhydrin color test based on this difference for screening high-lysine mutants of maize. The test also can be used for high-lysine sorghum and barley (1).

In this paper we present data on the free amino acid content of 14 maize

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**TABLE I**  
**Total Free Amino Acids in Single- and Double-Mutant Maize Endosperms (Inbred)**

Genotypes	Oh43		W64A		B37		C103	
	Lys. % <sup>a</sup>	Free amino acid <sup>b</sup>	Lys. %	Free amino acid	Lys. %	Free amino acid	Lys. %	Free amino acid
+ (Normal)	1.6	10.6	1.6	5.6	1.8	9.0	1.8	9.9
<i>o<sub>2</sub></i>	3.4	18.6	3.7	31.5	3.3	15.4	3.5	52.6
<i>fl<sub>2</sub></i>	2.5	16.0	3.0	16.2	2.8	7.9	2.6	12.2
<i>fl<sub>2</sub>o<sub>2</sub></i>	3.2	37.8	4.5	40.3	3.1	20.7	3.0	21.1
<i>ae</i>	2.2	5.9	2.0	2.4	2.2	6.7	2.2	7.2
<i>aeo<sub>2</sub></i>	3.8	17.8	4.5	37.7	3.8	15.6	3.6	69.7
<i>du</i>	2.0	11.3	1.8	6.7	2.1	9.0	2.0	12.7
<i>duo<sub>2</sub></i>	3.9	28.7	4.5	40.5	3.7	23.9	3.6	95.7
<i>su<sub>2</sub></i>	2.2	9.4	1.9	6.1	2.0	8.0	2.0	12.5
<i>su<sub>2</sub>o<sub>2</sub></i>	3.8	13.2	4.5	52.9	3.3	22.8	3.4	32.4
<i>wx</i>	1.7	8.8	1.7	7.5	2.0	14.1	1.7	11.2
<i>wxo<sub>2</sub></i>	3.9	21.4	4.3	49.6	3.8	22.1	3.1	104.8

<sup>a</sup>g lysine/100 g protein.

<sup>b</sup> $\mu$ M leucine/100 mg protein. Range of total protein ( $N \times 6.25$ ) = 8.6–12.2%.

*Opaque-2* mutants underlined.

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endosperm mutants and their double-mutant combinations with  $o_2$  in four inbred lines, as well as certain hybrid combinations of these inbreds (2,3,4).

In all combinations studied, the presence of the  $o_2$  gene invariably raised the level of free amino acids (as measured with ninhydrin) above that of the single mutant, inbred, or hybrid line.

TABLE II  
Total Free Amino Acids in Single- and Double-Mutant Maize Whole Kernel (Inbred)

Genotypes	Oh43	W64A	B37	C103
	Free amino acids <sup>a</sup>	Free amino acids	Free amino acids	Free amino acids
+ (Normal)	11.2	11.5	13.4	13.9
$o_2$	31.0	53.3	35.7	61.5
$o_1$	23.8	22.9	22.5	20.3
$o_1o_2$	39.9	...	...	...
$o_7$	52.3	35.0	76.6	147.5
$fl_1$	13.6	9.8	12.6	15.0
$fl_1o_2$	19.3	38.2	30.9	56.6
$fl_2$	25.0	30.3	24.7	23.9
$fl_2o_2$	40.1	52.1	42.5	51.9
$bt_1$	19.1	19.7	43.9	52.5
$bt_1o_2$	68.6	...	...	...
$bt_2$	35.6	35.2	43.8	35.6
$bt_2o_2$	47.1	...	...	...
$ae$	22.3	26.7	23.0	19.9
$aeo_2$	41.9	60.4	30.9	94.7
$du$	26.8	12.3	15.4	16.2
$duo_2$	40.3	55.7	29.9	104.7
$su_1$	20.5	26.1	22.6	23.6
$su_1o_2$	37.7	56.4	43.6	29.1
$su_2$	21.8	18.2	24.5	15.3
$su_2o_2$	30.3	66.2	36.8	48.0
$sh_1$	17.8	16.8	14.3	17.1
$sh_1o_2$	66.7	49.2	47.0	37.1
$sh_2$	24.7	27.4	37.5	106.1
$sh_2o_2$	41.8	66.1	51.2	...
$sh_4$	38.8	26.3	46.6	56.8
$sh_4o_2$	56.6	...	...	...
$h$	18.8	16.9	14.6	15.7
$ho_2$	28.3	26.6	26.5	58.7
$wx$	17.2	15.5	31.3	31.3
$wxo_2$	43.4	67.9	41.1	132.5

<sup>a</sup> $\mu$ M leucine/100 mg protein. Range of protein ( $N \times 6.25$ ) = 11.0–22.2%.

*Opaque-2* mutants underlined.

## MATERIALS AND METHODS

The endosperm mutants were from four inbred lines—Oh43, W64A, B37, and C103—each nearly isogenic, after six backcrosses, for one of the recessive genes *opaque-2* (*o<sub>2</sub>*), *floury-2* (*fl<sub>2</sub>*), amylose-extender (*ae*), dull (*du*), sugary-2 (*su<sub>2</sub>*), waxy (*wx*), sugary-1 (*su<sub>1</sub>*), shrunken-2 (*sh<sub>2</sub>*), floury-1 (*fl<sub>1</sub>*), soft-starch (*h*), shrunken-1 (*sh<sub>1</sub>*), their double recessive combinations with *o<sub>2</sub>*, and their normal counterparts. Certain genotypes from a diallel set of hybrids from these four inbred backgrounds were also evaluated. In addition, the mutants brittle-1 (*bt<sub>1</sub>*), brittle 2 (*bt<sub>2</sub>*), and shrunken-4 (*sh<sub>4</sub>*), in various stages of conversions to the four inbred lines and their double-mutant combinations with *o<sub>2</sub>*, were studied. These materials were grown in 1971 on the Purdue University Agronomy Farm and harvested at maturity.

All samples were pulverized and defatted. Lysine in the endosperms was determined in the acid hydrolysates with a Beckman amino acid analyzer. Nitrogen was estimated by the micro-Kjeldahl method.

## Quantitative Determination of Free Amino Acids

Ninhydrin reagent was prepared as described previously (1).

TABLE III  
Free Amino Acids in Hybrid Maize Whole Kernels<sup>a</sup>

Hybrids	Range Free Amino Acids <sup>b</sup>	Average Free Amino Acids
+	7.3–16.4	11.7
<i>o<sub>2</sub></i>	26.8–72.2	52.4
<i>fl<sub>2</sub></i>	19.3–37.7	25.3
<i>fl<sub>2</sub>o<sub>2</sub></i>	37.7–61.1	46.6
<i>ae</i>	12.4–18.3	14.7
<i>aeo<sub>2</sub></i>	28.1–81.7	62.8
<i>ae<sup>u</sup>fl<sub>2</sub></i>	20.8–36.3	28.8
<i>du</i>	14.6–23.5	19.1
<i>duo<sub>2</sub></i>	25.5–62.3	41.1
<i>wx</i>	11.6–14.7	13.2
<i>wxo<sub>2</sub></i>	(5 samples) 38.3–87.9	54.9
<i>wx<sup>u</sup>fl<sub>2</sub></i>	14.0–40.1	24.7
<i>su<sub>1</sub></i>	27.6–44.5	34.4
<i>su<sub>1</sub>o<sub>2</sub></i>	47.2–107.4	72.6
<i>su<sup>u</sup>fl<sub>2</sub></i>	18.5–39.2	30.1
<i>su<sub>2</sub></i>	(5 samples) 6.1–17.1	13.0
<i>su<sub>2</sub>o<sub>2</sub></i>	26.1–44.3	35.1
<i>sh<sub>2</sub></i>	22.3–58.3	35.0
<i>sh<sub>2</sub>o<sub>2</sub></i>	36.7–98.9	55.1

<sup>a</sup>Hybrids used are from a diallel among the four inbred lines Oh43, W64A, B37, and C103.

*Opaque-2* mutants underlined.

<sup>b</sup> $\mu$ M leucine/100 mg P. Range of protein ( $N \times 6.25$ ) = 11.7 to 18.7%.

One hundred milligrams of defatted ground samples of whole kernel or endosperm was suspended in 10 ml of distilled water. The contents were shaken at room temperature for 20 min, centrifuged at 12,000 rpm for 10 min, and the supernatant filtered if necessary. Three volumes of the supernatant (0.1, 0.15, and 0.2 ml in the present study) were placed in different test tubes and made to 0.5 ml with distilled water. Contents of the test tube were mixed well with 1.5 ml of ninhydrin reagent and heated in a boiling-water bath for 20 min. After cooling the tubes to room temperature, 8 ml of 50% *n*-propyl-alcohol was added, the mixture allowed to stand for 10 min, and the contents shaken on a Vortex mixer. Absorbance of the resulting solutions was read in a Spectronic-20 colorimeter at 570 nm, and the  $\mu\text{mol}$  equivalent of leucine present was determined by reference to a standard curve similarly prepared with solutions of L-leucine.

### RESULTS AND DISCUSSION

Table I shows the free amino acids in single- and double-mutant maize endosperms from four different inbred lines. Results indicate that  $o_2$  has a higher level of free amino acids in all the genetic backgrounds studied when compared with the respective normal counterpart of the same genotype. However, there are wide differences between inbred lines (two- to fivefold). Interestingly, in the case of the double mutants, introduction of  $o_2$  increases the free amino acid level of all of the single-mutant genotypes, again with wide differences between different inbreds.

Both lysine and free amino acids in the double mutants (Table I) are higher than in the corresponding single mutants. The lysine in the normal counterpart of various inbreds is quite similar, *i.e.*, 1.6–1.8%, but the free amino acids as  $\mu\text{M}$  leucine/100 mg protein vary from 5.6 in W64A to 10.6 in Oh43. The same is true with  $o_2$ , where the lysine is found in a narrow range of 3.3–3.7% in the four inbreds, but the free amino acids vary from 15.4  $\mu\text{M}$  leucine equivalents in B37 to 52.6  $\mu\text{M}$  leucine equivalents in C103. Because of these variations in free amino acid levels, the latter cannot be used to predict lysine levels.

Table II shows the free amino acid content of 111 single- and double-mutant whole kernel samples. Here also the double mutants show an increase in free amino acid level when compared with the single mutant of the same inbred genotype. It is of interest to note that the  $o_7$  mutant is exceptionally high in free amino acids in all inbreds tested, usually higher than  $o_2$  itself.

We extended our studies to various maize hybrids. Free amino acids were estimated (112 samples) on hybrid kernels, and their ranges and averages are tabulated (Table III). The data were calculated from six different samples. The  $o_2$  gene again increased the amount of free amino acids above that in the single-mutant hybrid. The  $fl_2$  gene had an intermediate effect.

It is obvious from these data that the introduction of the  $o_2$  gene into a wide variety of single mutants and normal genotypes, both inbred and hybrid, has a profound effect on the free amino acid metabolic pool in the endosperm.

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