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ACCUMULATION OF AMINO ACID NITROGEN AND ACID-HYDROLYZABLE AMMONIUM NITROGEN IN *OPAQUE-2* AND NORMAL MAIZE GRAIN

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ABSTRACT - Maize (*Zea mays* L.) plants of an *opaque-2* genotype (Pioneer L3369) and its normal counterpart (Pioneer 3369A) were grown in sand culture, using nutrient solution. During the first 36 days of reproductive growth, half of the plants of each genotype received no nitrogen and the other half received 3.75 m*M* N in nutritionally sufficient, modified Hoagland solution. Ears were harvested at 12, 24, and 36 days after pollination, and the amounts of nitrogen in each of 17 amino acid fractions and the acid-hydrolyzed ammonium fraction were determined. Using regression analysis and the first and second derivatives of the regression equations, amounts and rates of nitrogen accumulation were estimated for the amino acid and acid-hydrolyzed ammonium fractions of the grain. The *opaque-2* gene diminished the rates of accumulation of methionine (MET), tyrosine (TYR), isoleucine (ILE), phenylalanine (PHE), serine (SER), proline (PRO), alanine (ALA), and leucine (LEU) while increasing the rates of accumulation of lysine (LYS). The number of days after pollination at which the maximum rate of nitrogen accumulation occurred was earlier for the TYR, SER, PRO, ALA, LEU, and glutamate (GLU) fractions of the *opaque-2,* compared to the normal grain. Presence of the *opaque-2* gene tended to maintain almost constant ratios between the rates of nitrogen accumulation in LYS, THR, ILE, and MET fractions, compared to the nitrogen accumulation rates in the aspartate (ASP) fraction during the 36-day post-pollination period, while in the grain of the normal genotype, the ratios increased to a greater extent. From the ammonium N released during acid hydrolysis it was estimated that the GLU and ASP residues in the grain from the two genotypes were amidated to the same extent until about 20 days after pollination, but thereafter the percentage of amidated residues was greater in the 3369A.

KEY WORDS: Maize (*Zea mays* L.) grain, *opaque-2* gene, Aspartate family of amino acids, Nitrogen metabolism, Amino acids.

INTRODUCTION

During filling of maize grain, nitrogen for protein synthesis must be impor-

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ted as inorganic N from the root medium or as organic and inorganic N from metabolic or storage pools in other parts of the plant. In developing grain, the amino acid building blocks of proteins can be from *de novo* synthesis or be transported as such (ARRUDA and DA SILVA, 1979) from vegetative organs of the plant. It is likely that both processes would be subject to environmental influences and genetic factors, but few measurements of these effects on the overall rate of accumulation of specific amino acids have been reported. While recognizing that the values obtained would represent net changes and be the results of many dynamic processes, their interpretation in the light of established intermediary metabolism reactions can help elucidate mechanisms operative in whole plants during the reproductive phase of development.

The purpose of this study was to estimate rates of nitrogen accumulation in specific nitrogenous fractions, in particular, the amino acids and acid-hydrolyzable ammonium fraction of maize grain, as affected by a nutritional and a genetic factor. Composition of the grain of a commercial maize hybrid was compared with that of its counterpart containing the *opaque-2* gene during a 36-day period following pollination. Since the influence of the *opaque-2* gene on amino acid composition of maize grain was first reported in 1964 by MERTZ, BATES and NELSON, considerable research has been directed toward ascertaining its effects on physiological mechanisms. One set of plants consisting of both genotypes was provided a complete nutrient solution containing nitrate nitrogen during its entire period of growth. Another set was grown similarly, but no nitrogen was provided after pollination.

MATERIALS AND METHODS

Maize seeds of Pioneer L3369 (*opaque-2*) and Pioneer 3369A (normal) genotypes were germinated between paper towels in contact with 0.2 m*M* $CaSO_4$. Because of slower germination observed previously, L3369 was started 2 days prior to 3369A. The most uniform seedlings were selected and two of each genotype were transplanted in each of 24 pots containing 17.2 kg of washed sand. After becoming established, one of each genotype was removed. Until pollination, all pots were watered daily with a complete nutrient solution, with some adjustments in concentration of specific nutrients as indicated by plant growth. In the period prior to pollination all pots were being watered with a solution essentially similar to that of a so-called «Johnson solution» (EPSTEIN, 1972). Open pollination was prevented by covering the tassels and silks as they appeared, and the plants were hand-pollinated. Beginning at the time of pollination, one set of plants was watered with nutrient solution containing 3.75 m*M* NO_3-N. The other set was watered with a similar solution which contained no nitrogen.

A total of 24 pots was used, four of each set to be harvested at 12, 24, and 36 days after pollination. The ears were frozen and the grain subsequently shelled in the cold. Leaves below the ears were dried at 70° C, then finely ground, and total nitrogen was determined by the Kjeldahl method (A.O.A.C., 1960). Grain samples were lyophilized, finely ground, and hydrolyzed with 5.7 *N* HC1 *in vacuo* at 108° C for 48 hours. The HC1 was removed under reduced pressure in a N_2 atmosphere at 60° C (RENDIG and BROADBENT, 1979). Aliquots were lyophilized, buffer was added, and quantitative analysis of the amino acids and ammonium in the hydrolyzate from the grain proteins, with the exception of cysteine and tryptophan, was done with a Durrum D-500 automatic amino acid analyzer. Tryptophan was determined separately on samples which were defatted with petroleum ether (Ligroine 30°-60°) and were then hydrolyzed for 10 hours at 120° C in 5 *N* NaOH. The hydrolyzates were neutralized, and tryptophan was absorbed on Dowex-50 (NH_4^+ form) resin, eluted, and determined colorimetrically (DICKMAN and CROCKETT, 1956).

Analysis of variance (AOV) was performed on the amount (mg) of N, per plant, for each amino acid fraction and for the acid-hydrolyzed ammonium fraction of the grain from each sampling. For AOV, the model used was $y = A+B+AB$, where y = amount of N in the biochemical fraction, A = N treatment main effect, B = genotype main effect, and AB = effect due to the interaction of N treatment and genotype. There were 16 cases of a main effect of genotype and 4 instances of a main effect of nitrogen treatment (Table 1). Data from the three samplings showed no significant ($P = 0.05$) interaction of N treatment and genotype.

On the basis of the AOV, individual plant measurements for the amount of N in each of the biochemical fractions of the grain per plant were pooled from both the 0 and 3.75 mM N treatments, and parameters of regression equations were then calculated. The purposes for developing regression equations from the data are 1) to provide a means of estimating the amount of N in each fraction of the grain, 2) to estimate the rate of accumulation of N in each fraction by the first derivative of the regression equation, and 3) to estimate the day on which the maximum rate of N accumulation occurred in each fraction, using the second derivative of the regression equation, during the 36-day period following pollination. In an initial test, it was found that normal cubic equations described the data better than did either normal quadratic or linear equations, as indicated by the coefficient of determination, R^2, the fraction of the total sum of squares due to regression. The complete cubic polynomial and its first derivative may be expressed as:

$$y = \beta_0+\beta_1x+\beta_2x^2+\beta_3x^3 \quad \text{and} \quad y' = \beta_1+2\beta_2x+3\beta_3x^2.$$

However, it was decided not to use the normal cubic polynomials because of two shortcomings. Firstly, they estimated negative amounts of nitrogen in the biochemical fractions of the grain during the first two weeks after pollination, and secondly, their first derivatives estimated rates of accumulation of N other than zero at the time of pollination and net efflux of N from the biochemical fractions during approximately the first week after pollination. It is physically impossible to have negative amounts of N, and net efflux of N from any of the biochemical fractions under study is highly unlikely during the early stages of grain development. To correct these shortcomings of the normal cubic polynomials, we specified that $y = 0$ when $x = 0$ and $y' = 0$ when $x = 0$, yielding a new model with which to fit the data: $y = \beta_2x^2 + \beta_3x^3$.

For an observation from an individual plant,

$$y_i = \beta_2x_i^2 + \beta_3x_i^3 + \varepsilon_i,$$

where ε_i = the vertical deviation of y_i from the regression line. Moreover, when fitting the cubic polynomial of two terms to a group of paired variates (x_i, y_i),

$$Q = \sum_{i=1}^{n} (y_i - \beta_2 x_i^2 - \beta_3 x_i^3)^2,$$

where Q equals the sum of squares of vertical deviations from the regression line by data for which the regression line provides estimates as functions of the independent variable. Setting the first partial derivatives of Q with respect to b_2 and b_3, the estimates of β_2 and β_3, equal to zero,

$$\frac{\delta Q}{\delta b_2} = -2 \sum_{i=1}^{n} x_i^3 (y_i - b_2 x_i^2 - b_3 x_i^3) = 0, \text{ and}$$

$$\frac{\delta Q}{\delta b_3} = -2 \sum_{i=1}^{n} x_i^2 (y_i - b_2 x_i^2 - b_3 x_i^3) = 0$$

These two equations yield the following two equations which, for each biochemical fraction of *opaque-2* or normal grain, were solved simultaneously for b_2 and b_3:

$$(\Sigma x_i^4) b_2 + (\Sigma x_i^5) b_3 = \Sigma x_i^2 y_i$$
$$(\Sigma x_i^5) b_2 + (\Sigma x_i^6) b_3 = \Sigma x_i^3 y_i$$

This method of determining b_2 and b_3 for the regression equations estimating mg amino acid N or mg acid-hydrolyzed ammonium N as a function of time provided cubic functions with R^2 values generally greater than 0.90. The first and second derivatives of $y = b_2x^2 + b_3x^3$ are:

$$y' = 2b_2x + 3b_3x^2 \text{ and } y'' = 2b_2 + 6b_3x.$$

The first derivative of each regression equation was calculated, and curves were drawn, providing continuous estimates of the rate of nitrogen accumulation in each fraction for the grain of each genotype. The second derivative of each regression equation was calculated and each was solved for x, with y" equaling zero, to estimate the day of the maximum rate of N accumulation during the 36-day experimental period for each fraction.

RESULTS AND DISCUSSION

The mean concentrations of nitrogen in the lower leaves of the normal (3.05% N) and *opaque-2* (2.85% N) maize plants at silking suggest that the plants were sufficiently supplied during vegetative growth with enough nitrogen to attain maximum grain yield. Recent research (RENDIG and AMPARANO, 1980) using Pioneer 3369A and Pioneer L3369 genotypes - the same as used in the present study - showed that plants of these genotypes having at least 2.53% N in the lower leaves at silking achieved maximum grain yields in the greenhouse. In the present study, mean grain yields per plant 36 days after pollination were 124 g for the normal genotype and 104 g

TABLE 1 - *Amounts (mg) of amino acid N and acid-hydrolyzable ammonium N in grain per maize plant at samplings for which there were significant differences ($P \leq 0.05$) due to nitrogen treatment or genotype of the grain.*

Nitrogen Fraction	Days after Pollination	N Treatment (m*M*)		Genotype (1)		P(2)
		0	3.75	Normal	*Opaque-2*	
	24	12.2	16.6	—	—	**
Methionine	24	—	—	16.4	12.4	*
	36	18.8	25.2	—	—	*
	24	19.6	24.2	—	—	*
Tyrosine	24	—	—	25.8	18.0	**
	36	—	—	46.8	33.0	**
Isoleucine	24	—	—	34.8	26.4	**
	36	—	—	58.8	45.7	*
Phenylalanine	24	—	—	33.0	21.9	***
	36	—	—	57.6	38.8	***
Serine	24	—	—	57.4	44.9	*
	36	—	—	97.7	75.9	*
Lysine	36	—	—	72.6	86.6	**
Proline	24	—	—	92.3	67.1	**
	36	—	—	157.4	118.8	*
Alanine	24	—	—	116.4	91.1	*
	36	—	—	192.0	146.4	**
Leucine	24	—	—	113.3	69.8	***
	36	—	—	203.6	122.7	***
Ammonium	12	30.6	42.2	—	—	*

(1) Genotypes are normal (Pioneer 3369A) and *opaque-2* (Pioneer L3369).

(2) *** Significance at $P = 0.001$; ** Significance at $P = 0.01$; * Significance at $P = 0.05$.

for its *opaque-2* counterpart; these mean yields do not differ significantly ($P = 0.10$). The amount of nitrogen in the grain 36 days after pollination does differ significantly ($P = 0.05$) between the *opaque-2* and normal genotypes - 2222 mg N in the normal and 1863 mg N in the *opaque-2* grain per plant.

At 24 and 36 days after pollination, the grain from the *opaque-2* plants was found to differ significantly ($P = 0.05$) from that of the normal counterpart in the accumulation of nitrogen in nine amino acid fractions (Table 1). Differences were also detected for amounts of nitrogen in methionine, tyrosine, and acid-hydrolyzed ammonium fractions of the whole grain,

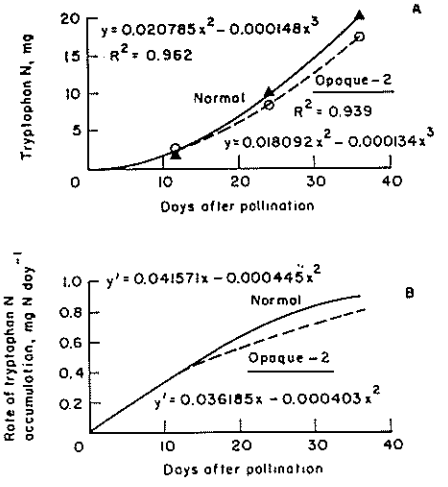

FIGURE 1. Amounts (A) and rates (B) of tryptophan nitrogen accumulation in normal (Pioneer 3369A) and *opaque-2* (Pioneer L3369) maize grain per plant. Symbols represent means of replicates pooled from 0 and 3.75 m*M* N treatments.

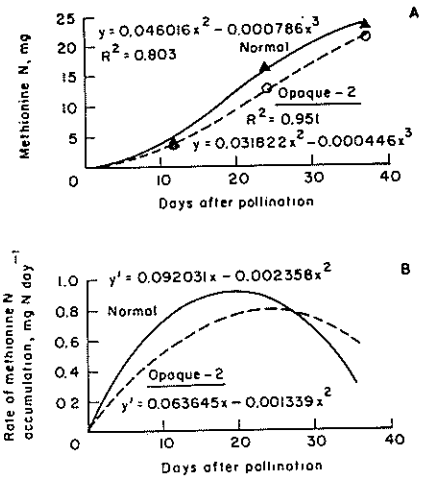

FIGURE 2. Amounts (A) and rates (B) of methionine nitrogen accumulation in normal (Pioneer 3369A) and *opaque-2* (Pioneer L3369) maize grain per plant. Symbols represent means of replicates pooled from 0 and 3.75 m*M* N treatments.

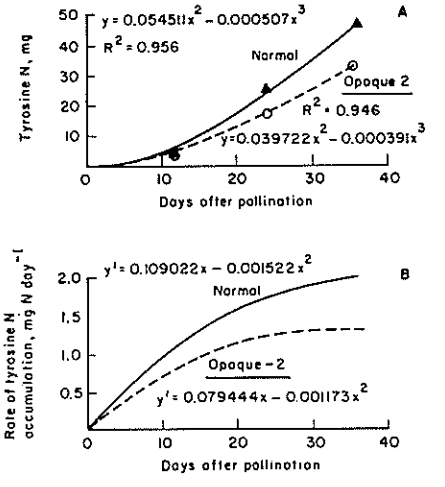

FIGURE 3. Amounts (A) and rates (B) of tyrosine nitrogen accumulation in normal (Pioneer 3369A) and *opaque-2* (Pioneer L3369) maize grain per plant. Symbols represent means of replicates pooled from 0 and 3.75 m*M* N treatments.

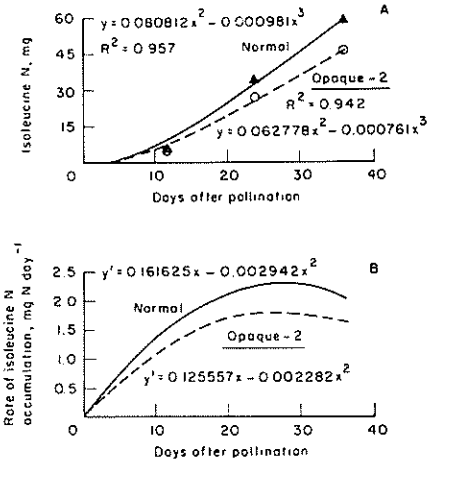

FIGURE 4. Amounts (A) and rates (B) of isoleucine nitrogen accumulation in normal (Pioneer 3369A) and *opaque-2* (Pioneer L3369) maize grain per plant. Symbols represent means of replicates pooled from 0 and 3.75 m*M* N treatments.

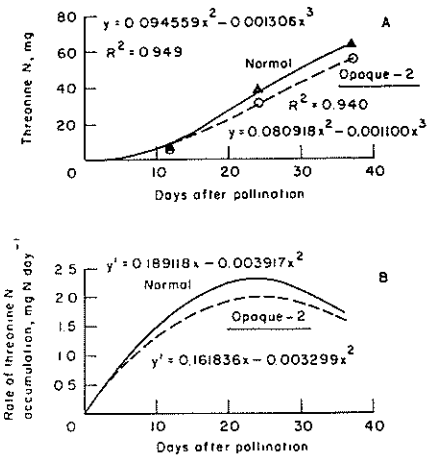

FIGURE 5. Amounts (A) and rates (B) of threonine nitrogen accumulation in normal (Pioneer 3369A) and *opaque-2* (Pioneer L3369) maize grain per plant. Symbols represent means of replicates pooled from 0 and 3.75 m*M* N treatments.

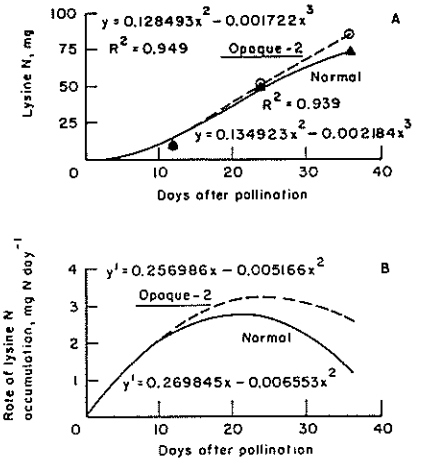

FIGURE 6. Amounts (A) and rates (B) of lysine nitrogen accumulation in normal (Pioneer 3369A) and *opaque-2* (Pioneer L3369) maize grain per plant. Symbols represent means of replicates pooled from 0 and 3.75 m*M* N treatments.

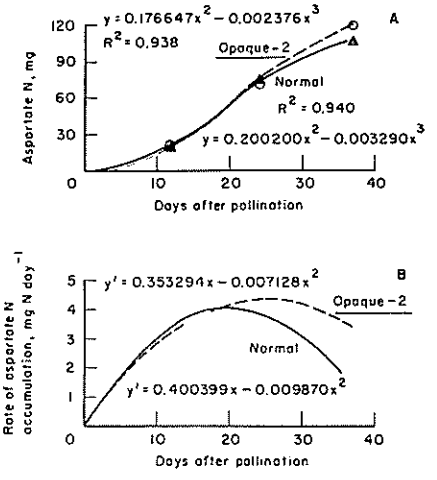

FIGURE 7. Amounts (A) and rates (B) of aspartate nitrogen accumulation in normal (Pioneer 3369A) and *opaque-2* (Pioneer L3369) maize grain per plant. Symbols represent means of replicates pooled from 0 and 3.75 m*M* N treatments.

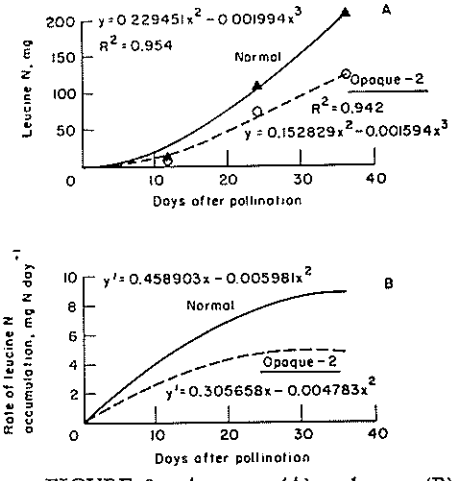

FIGURE 8. Amounts (A) and rates (B) of leucine nitrogen accumulation in normal (Pioneer 3369A) and *opaque-2* (Pioneer L3369) maize grain per plant. Symbols represent means of replicates pooled from 0 and 3.75 m*M* N treatments.

due to presence or absence of nitrogen in the nutrient solution provided to the plants after pollination. In Figures 1 through 10, the amounts and rates

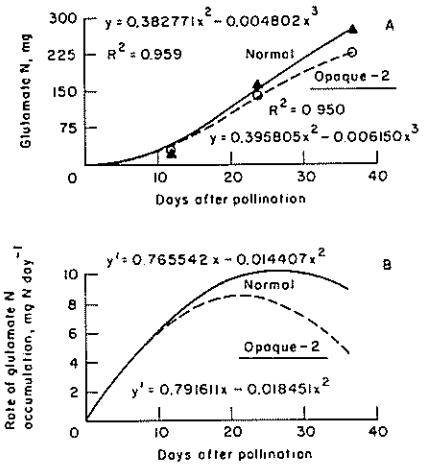

FIGURE 9. Amounts (A) and rates (B) of glutamate nitrogen accumulation in normal (Pioneer 3369A) and *opaque-2* (Pioneer L3369) maize grain per plant. Symbols represent means of replicates pooled from 0 and 3.75 m*M* N treatments.

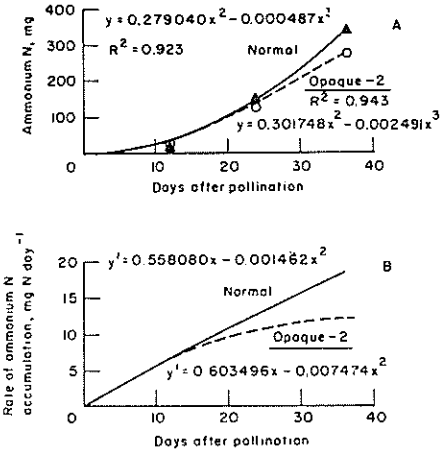

FIGURE 10. Amounts (A) and rates (B) of hydrolyzable ammonium nitrogen accumulation in normal (Pioneer 3369A) and *opaque-2* (Pioneer L3369) maize grain per plant. Symbols represent means of replicates pooled from 0 and 3.75 m*M* N treatments.

of N accumulation in selected amino acid fractions and in the hydrolyzed ammonium fraction of normal and *opaque-2* grain are arranged sequentially from the fraction containing the least amount of nitrogen at 36 days after pollination (tryptophan N, Fig. 1) to the fraction containing the greatest amount of N at the 36-day sampling (hydrolyzed ammonium N, Fig. 10).

Rates of nitrogen accumulation during the 36-day post-pollination period were least for the tryptophan (Fig. 1) and methionine (Fig. 2) fractions. Interestingly, among the deoxyribonucleic acid (DNA) codons which specify the sequences of amino acids in proteins, there are only one each coding for these two amino acids. In contrast, arginine and leucine (Fig. 8), for example, are specified by six codons each (LAGERKVIST, 1980). The rates of methionine N accumulation in the grain increased until about 20 days after pollination in the normal grain and 24 days after pollination in the *opaque-2* grain, then began decreasing (Table 2, Fig. 2), indicating a repression in methionine synthesis or possibly a reduction in the rate of influx of methionine to the developing grain at about 20 days after pollination. As noted earlier, the rates are calculated from net changes in amounts and do not distinguish nitrogen of amino acids which were synthesized in the grain from the N of those which were translocated to the grain from other parts of the plant.

TABLE 2 - *Number of days after pollination when maximum rate of nitrogen accumulation occurred in amino acid and acid-hydrolyzable ammonium fractions of maize grain during the first 36 days post-pollination.*

Nitrogen Fraction	Genotype (1)	
	Normal	*Opaque-2*
Tryptophan	(2)	(2)
Methionine	20	24
Tyrosine	(3)	34
Isoleucine	27	28
Phenylalanine	32	32
Threonine	24	25
Serine	28	26
Glycine	23	23
Valine	25	25
Lysine	21	25
Histidine	29	30
Aspartate	20	25
Proline	28	27
Alanine	23	21
Arginine	24	27
Leucine	(2)	32
Glutamate	27	21
Ammonium	(2)	(2)

(1) Genotypes are normal (Pioneer 3369A) and *opaque-2* (Pioneer L3369).
(2) Using second derivative of regression equation, when $y'' = 0, x > 36$.
(3) Using second derivative of regression equation, when $y'' = 0, x = 36$.

From the data (Table 1), it appears that nitrogen deprivation after pollination and the *opaque-2* gene were both effective in decreasing rates of accumulation of methionine in the developing maize grain.

The pattern of increase, followed by decrease, of the rates of accumulation of methionine N (Fig. 2) is in contrast to the patterns of rates of increase of tyrosine N (Fig. 3). During the 36-day experimental period, the rate of tyrosine N accumulation continually increased in the grain of the normal genotype, but reached a maximum 34 days after pollination (Table 2) in the *opaque-2* grain. This pattern of tyrosine N accumulation also differs from that of tryptophan (Fig. 1) where both normal and *opaque-2* grain continued to accumulate tryptophan N at increasingly greater rates during the 36-day period (Table 2). The reductions in the amount of tyrosine and another aromatic amino acid, phenylalanine, at 24 and 36 days after pollination due to the *opaque-2* gene (Table 1) are quantitatively greater but qualitatively similar to reductions in the amounts of these amino acids reported for mature,

whole grain of R802, WF9 × M14, and R80 × R75 maize (SODEK and WILSON, 1971).

Amounts of nitrogen accumulated in three aliphatic amino acid fractions - isoleucine, alanine, and leucine - were significantly ($P = 0.05$) diminished by the *opaque-2* gene at 24 and 36 days after pollination (Table 1). Since leucine (Fig. 8) and alanine are important components of the prolamin zein (SODEK and WILSON, 1971), the lower amounts of nitrogen in these two fractions of the *opaque-2* grain probably indirectly reflect a decrease in the rate of synthesis of zein. The production of any amino acid in the grain is, to some extent, regulated to correspond to demands for the amino acid for protein synthesis and other reactions (NELSON, 1970).

At 24 and 36 days after pollination, amounts of proline N and serine N were significantly ($P = 0.05$) less in the *opaque-2* grain, compared to the normal grain (Table 1). Like leucine, proline is a major constituent of zein (SODEK and WILSON, 1971), and the reduced accumulation of proline resulted from reduced proline synthesis, since proline is one of the two protein amino acids not translocated to developing maize grain (ARRUDA and DA SILVA, 1979). Although isoleucine and serine represent relatively small components of zein (SODEK and WILSON, 1971), their diminished rates of accumulation in the *opaque-2* grain are probably also a secondary effect of reduced zein synthesis.

Lysine, as expected (MERTZ, BATES and NELSON, 1964), was present in significantly greater amounts in the *opaque-2* grain, compared to the grain of the normal counterpart (Table 1, Fig. 6). At 36 days after pollination the percentage of total grain nitrogen as lysine was significantly ($P = 0.01$) greater in the *opaque-2* grain, compared to the normal grain. The estimated rates of accumulation of lysine N in the grain (Fig. 6) are essentially identical until about 12 days after pollination when the accumulation of lysine in the *opaque-2* grain becomes more rapid than that in the normal. It is evident from the data of the present experiment (Fig. 6) that lysine continued to accumulate at increasingly greater rates in the grain of both genotypes until reaching maximum rates at 21 and 25 days after pollination in the normal and *opaque-2* grain, respectively (Table 2), after which the rates declined - more rapidly in the normal grain than in the *opaque-2* grain.

The mass of nitrogen accumulated in the grain, per plant, as threonine (Fig. 5), glycine, valine, histidine, aspartate (Fig. 7), arginine, and glutamate (Fig. 9) N was not significantly ($P = 0.05$) affected by either nitrogen treatment or by the *opaque-2* gene on the 12th, 24th, or 36th day of reproductive

growth. Other experimental conditions may have resulted in significant differences in amounts of N accumulated in these amino acid fractions.

It is particularly noteworthy that the maximum rate of nitrogen accumulation in most of the biochemical fractions under scrutiny occurred betweeen 20 and 36 days after pollination in the grain of both genotypes (Table 2). The maximum rate of nitrogen accumulation occurred earlier in the methionine, isoleucine, threonine, lysine, histidine, aspartate, and arginine fractions of the normal grain, compared to the time of the maximum rate of accumulation of N in these fractions in the *opaque-2* grain. On the other hand, maximum rates of N accumulation in the tyrosine, serine, proline, leucine, and glutamate fractions were detected to occur earlier in the *opaque-2* than in the normal grain. For tryptophan, phenylalanine, glycine, valine, and acid-hydrolyzed ammonium fractions, there was no difference in the day of

TABLE 3 - *Amounts (mg) of nitrogen accumulated in the aspartate, lysine, threonine, isoleucine, and methionine fractions of normal (Pioneer 3369A) and* opaque-2 *(Pioneer L3369) maize grain per plant.*

	ASP		LYS		THR		ILE		MET	
DAP (1)	Nor.	*o2*	Nor.	*o2*	Nor.	*o2*	Nor.	*o2*	Nor.	*o2*
12	23.1	21.3	15.6	15.5	11.4	9.8	9.9	7.7	5.3	3.8
18	45.7	43.4	31.0	31.6	23.0	19.8	20.5	15.9	10.3	7.7
24	69.8	68.9	47.5	50.2	36.4	31.4	33.0	25.6	15.6	12.2
30	91.4	94.8	62.5	69.1	49.8	43.1	46.2	36.0	20.2	16.6
36	106.0	118.3	73.0	86.2	61.6	53.5	59.0	45.8	23.0	20.4

(1) DAP = Days after pollination; Nor. = Normal; *o2* = *Opaque-2;* ASP = Aspartate; LYS = Lysine; THR = Threonine; ILE = Isoleucine; MET = Methionine. Amounts were calculated using regression equations.

the maximum rate of accumulation of nitrogen in the grain, comparing the two genotypes during the 36-day experimental period.

Since the slopes of the first derivative curves for tryptophan N (Fig. 1) and acid-hydrolyzed ammonium N (Fig. 10) are only positive during the 36-day period, it appears that the rates of accumulation of N in these fractions probably continued to increase beyond this time before declining. For leucine (Fig. 8), the N accumulation rate continued to increase throughout the 36-day experimental period for the normal grain, but in the *opaque-2* grain leucine N accumulated at increasingly greater rates util 32 days after pollination when the maximum rate was reached, followed by declining rates of N accumulation.

In the aspartic acid family of amino acids, aspartate is a precursor in the biosynthesis of methionine, threonine, isoleucine and lysine (BRYAN, 1980). In maize kernels, lysine at certain concentrations is known to inhibit the enzymatically catalyzed conversion of aspartate to β-aspartophosphate, a precursor of lysine itself as well as methionine, threonine and isoleucine (GEGENBACH *et al.*, 1976). Threonine at certain concentrations can inhibit the enzymatically catalyzed synthesis of homoserine, a precursor of methionine, threonine, and isoleucine (GEGENBACH *et al.*, 1976). Data from the present experiment show the amounts of nitrogen (Table 3) and rates of nitrogen accumulation (Table 4) in the amino acid fractions of the aspartic acid family

TABLE 4 - *Rates of nitrogen accumulation (mg N day*$^{-1}$*) in the aspartate, lysine, threonine, isoleucine, and methionine fractions of normal (Pioneer 3369A) and* opaque-2 *(Pioneer L3369) maize grain per plant.*

	ASP		LYS		THR		ILE		MET	
DAP (1)	Nor.	*o2*	Nor.	*o2*	Nor.	*o2*	Nor.	*o2*	Nor.	*o2*
12	3.38	3.21	2.29	2.34	1.70	1.47	1.52	1.18	0.76	0.57
18	4.01	4.05	2.73	2.95	2.14	1.84	1.96	1.52	0.89	0.71
24	3.92	4.37	2.70	3.19	2.28	1.98	2.18	1.70	0.85	0.75
30	3.12	4.18	2.20	3.06	2.15	1.88	2.20	1.71	0.64	0.75
36	1.62	3.48	1.22	2.56	1.73	1.55	2.00	1.56	0.26	0.56

(1) DAP = Days after pollination; Nor. = Normal; *o2* = *Opaque-2*; ASP = Aspartate; LYS = Lysine; THR = Threonine; ILE = Isoleucine; MET = Methionine. Rates were calculated using first derivative of regression equations.

of amino acids, as calculated using regression equations and their first derivatives. More aspartate N and lysine N were accumulated in the *opaque-2* grain toward the latter part of the 36-day period of reproductive growth, compared to the normal grain. In contrast, more threonine N, isoleucine N and methionine N were accumulated in the normal grain, compared to the *opaque-2* grain, as time progressed.

The presence of the *opaque-2* gene in the L3369 maize tends to maintain almost constant ratios of N accumulation rates in lysine, threonine, isoleucine and methionine fractions, compared to the N accumulation rates in the aspartate fraction (Table 5), while in normal grain the ratios for lysine, threonine, and isoleucine, but not methionine, increase to a greater extent during grain development. Several factors are probably significant in controlling such ratios of the rates of N accumulation within the aspartic acid family of amino acids. Firstly, feedback inhibition is a well-known and relatively

TABLE 5 - *Ratios of N accumulation rates in lysine, threonine, isoleucine, and methionine fractions to N accumulation rates in aspartate fraction of normal (Pioneer 3369A) and* opaque-2 *(Pioneer L3369) maize grain* (1).

Days after pollination	LYS/ASP		THR/ASP		ILE/ASP		MET/ASP	
	Nor.	*o2*	Nor.	*o2*	Nor.	*o2*	Nor.	*o2*
12	0.68	0.73	0.50	0.46	0.45	0.37	0.22	0.18
18	0.68	0.73	0.53	0.45	0.49	0.38	0.22	0.18
24	0.69	0.73	0.58	0.45	0.56	0.39	0.22	0.17
30	0.71	0.73	0.69	0.45	0.71	0.41	0.21	0.18
36	0.75	0.74	1.07	0.45	1.23	0.45	0.16	0.16

	(LYS+THR+ILE+MET)/ASP		(THR+ILE+MET)/ASP		(THR+ILE)/ASP	
	Nor.	*o2*	Nor.	*o2*	Nor.	*o2*
12	1.86	1.73	1.18	1.00	0.95	0.83
18	1.93	1.73	1.24	1.00	1.02	0.83
24	2.04	1.74	1.35	1.01	1.14	0.84
30	2.30	1.77	1.60	1.04	1.39	0.86
36	3.22	1.99	2.46	1.05	2.30	0.89

(1) Nor.=Normal; *o2*=*Opaque-2;* ASP=Aspartate; LYS=Lysine; THR=Threonine; ILE=Isoleucine; MET=Methionine. Rates were calculated using first derivative of regression equations.

direct control of the biosynthesis of the four protein amino acids which have aspartate as their precursor (GEGENBACH *et al.*, 1976; BRYAN, 1980). The concentrations of the lysine, threonine, isoleucine, and methionine as free amino acids in the endosperm are critical factors regulating their synthesis in maize grain (GEGENBACH *et al.*, 1976). The concentrations of these free amino acids in the endosperm at any time during development depends upon a balance between 1) rates of import of the amino acids via the plant's vascular system to the grain, 2) rates of synthesis of the amino acids in the grain, 3) rates of removal of these amino acids from the free amino acid pool by their incorporation into proteins and 4) rates of removal of these amino acids from the free amino acid pool by conversion to other compounds, including amino acids, during grain development.

It appears from the data (Table 5) that in the *opaque-2* grain there is tight control at the trunk of the biosynthetic pathways in the aspartic acid family of amino acids, since the ratios of N accumulation remain almost constant, regardless of which amino acids in the family are chosen for comparison of rates of N accumulation to the rates of N accumulation in the precursor aspartate fraction. The primary effect of the *opaque-2* gene

has been shown to be the reduction of synthesis of zein, particularly during the first 20 days after pollination (JONES *et al.*, 1977), and secondary effects are the syntheses of greater amounts of non-zein proteins (MERTZ *et al.*, 1964; CRAWFORD, 1980). Since zein was synthesized at reduced rates and since other proteins were synthesized at greater rates in the *opaque-2* grain (CRAWFORD, 1980), it is highly probable that the rates of synthesis of these proteins are the primary factors in determining the rates of accumulation of the members of the aspartic acid family of amino acids. Secondary regulation of the syntheses of the amino acids of the aspartic acid family in maize grain occurs, therefore, by feedback inhibition of enzymatic catalysis of the biosynthetic reactions which ultimately require aspartate as a precursor.

During acid hydrolysis, the breakdown of some amino acids, possibly threonine and serine (SMYTH *et al.*, 1962), could account for some of the ammonium N found in the hydrolyzates. Under the conditions used in the present study, however, it is likely that most of this fraction is amide N from asparagine and glutamine, amino acids which could be present both in the free amino acid pool and as components of proteins of the grain. With this assumption, the extent of amidation of the aspartate and glutamate residues was estimated for 10, 20, 30, and 36 days after pollination using regression equations (Table 6). Until about 20 days after pollination the

TABLE 6 - *Amounts (mg) of nitrogen accumulated in the aspartate, glutamate and acid-hydrolyzed ammonium fractions of normal (Pioneer 3369A) and* opaque-2 *(Pioneer L3369) maize grain per plant.*

Genotype	Days after pollination	Aspartate	Glutamate	Ammonium	Percent (1) Amidated
Normal	10	16.7	33.5	27.4	55
	20	53.8	114.7	107.7	64
	30	91.4	214.8	238.9	78
	36	106.0	272.0	338.9	89
Opaque-2	10	15.3	33.4	27.7	57
	20	51.6	109.1	100.8	63
	30	94.8	190.2	204.3	72
	36	118.1	226.0	274.8	80

(1) Percentage of the sum of the aspartate and glutamate residues which was amidated, assuming all acid-hydrolyzed ammonium nitrogen to be amide nitrogen. Amounts were calculated using regression equations.

percentage of amidation was very similar in the grain of both genotypes, but

thereafter, greater values were found for the normal grain. Differences in amide ammonia content of several biochemical fractions separated by Cu fractionation from *opaque-2* and normal maize grain endosperm were reported by MERTZ and his colleagues (1964). A greater amount of amide ammonia was found in the « acid-soluble » fraction of normal endosperm taken from a single backcross ear, compared to the same fraction of *opaque-2* endosperm from the same ear. The reverse was true in the « zein » fraction, while for the « glutelin » fraction the amide ammonia values for the two genotypes were similar.

By the use of regression analysis and first and second derivatives of the regression equations, hitherto unrecognized patterns in rates of nitrogen accumulation in amino acid and acid-hydrolyzed ammonium fractions of two genotypes of maize grain have been shown. The potential uses of differential equations in describing dynamic phenomena in plant physiology are many; rates of nitrogen accumulation in biochemical fractions of maize grain are but one example.

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RIASSUNTO

Accumulo di azoto aminoacidico e di ammonio nella granella di mais opaco-2 *e normale*

Le versioni normale e *opaco-2* dell'Ibrido Pioneer 3369 sono state coltivate su sabbia utilizzando una soluzione nutritiva. Nei primi 36 giorni dello sviluppo riproduttivo metà delle piante di entrambi i genotipi non ha ricevuto azoto; l'altra metà ha ricevuto una soluzione Hoagland-modificata 3,75 m*M* in azoto. Le spighe sono state raccolte a 12, 24 e 36 giorni dall'impollinazione; si sono poi determinati i livelli dei 17 aminoacidi e della frazione di ammonio idrolizzabile. La velocità di accumulazione e la quantità accumulata dei 17 aminoacidi e dell'ammonio sono state stimate utilizzando le equazioni di regressione ottenute interpolando i dati sperimentali. Il gene *opaco-2* riduce le velocità di accumulo di metionina, tirosina, isoleucina, fenilalanina, serina, prolina, alanina e leucina ed aumenta quelle della lisina. Nel materiale *opaco-2* la velocità massima di accumulo è stata raggiunta prima, nei confronti della versione normale, per tirosina, serina, prolina, alanina, leucina e acido glutammico. In presenza del gene *o2* si mantengono costanti i rapporti tra le velocità di accumulo dell'azoto nella lisina, treonina, isoleucina e metionina confrontate a quella dell'acido aspartico, mentre nel seme del mais normale i rapporti aumentano. A partire dall'azoto idrolizzato in ambiente acido si è stimato che, in entrambi i genotipi, i residui degli acidi glutammico e aspartico erano amidati allo stesso livello fino a 20 giorni dopo l'impollinazione; in seguito il grado di amidazione è più elevato nella versione normale.

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