STUDIES ON CORN PROTEINS. XI. DISTRIBUTION OF LYSINE DURING GERMINATION OF NORMAL AND *Opaque*-2 MAIZE[1]

B. A. K. CHIBBER, E. VOICU[2], E. T. MERTZ, Department of Biochemistry, and D. V. GLOVER, Department of Agronomy, Purdue University, West Lafayette, IN 47907

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ABSTRACT

Levels of total and free (pool) lysine in germinating normal [(Oh43+ × B37+) × C103+] and *opaque*-2 [(Oh43o_2 × B37o_2) × C103o_2] maize were monitored over an 11-day period. Germination at 28°C in the dark (without supplemental carbon or nitrogen) was accompanied by an increase in total and free lysine levels in normal maize seedlings while, in *opaque*-2 seedlings, total lysine declined over an 11-day period, accompanied by an increase in the free lysine pool. Total nitrogen content remained constant in both experiments, while there was a 15% loss in total dry weight over the 11-day period. Both normal and *opaque*-2 seedlings developed comparable levels of aspartokinase activity which reached a maximum at 10 days post germination. Aspartokinase preparations from both normal and high-lysine maize appeared to be subject to feedback inhibition by lysine.

The discovery of high-lysine mutants of maize (1–3), followed by those of barley (4,5) and sorghum (6), has generated considerable speculation concerning the biochemical mechanism(s) responsible for the high-lysine genotype. Detailed developmental studies have been conducted on a variety of high-lysine maize mutants from pollination to maturity (7,8). These have strongly suggested that the mutation(s) primarily affect developmental process(es) during seed maturation. Extensive studies on a large number of high-lysine mutants have characterized the major biochemical and morphological phenotypes associated with the high-lysine genotype(s) in maize (9–12). High-lysine maize is associated with a decrease in its content of zein, the major storage protein in corn (10), malformation of protein bodies (11), and an increase in the level of free amino acids (12). The decrease in zein content is offset, to varying degrees in different mutants, by an increase in the content of albumins, globulins, and glutelins in mature seeds. In developing high-lysine maize seeds, the decrease in zein content occurs early in development after pollination, primarily due to a lack in zein synthesis (7,8). Recently, it has been suggested that high-lysine mutants of maize may have a mutant β-aspartokinase (13). This enzyme plays a key regulatory role in the biosynthesis of lysine, threonine, methionine, and isoleucine (14), catalyzing the committed step in their biosynthesis by converting aspartic acid to β-aspartyl phosphate. The activity of its various forms is subject to multiple and concerted feedback inhibition by lysine, threonine, and methionine. The proposition that a mutant aspartokinase, free from feedback control of its activity, might cause elevated levels of lysine in maize is based upon germination and developmental studies on isolated corn embryos in culture (13). In this report, we describe the effects of the high-lysine gene on lysine biosynthesis during germination of maize.

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[2]CIMMYT-UNDP Trainee. Present address: Laboratory of Biochemistry I.C.C.P.T. Fundulea, Bucarest, Romania.

MATERIALS AND METHODS

Seeds of a near-isogenic three-way hybrid homozygous for the high-lysine mutant *opaque*-2 [(Oh43o_2 × B37o_2) × C103o_2] and its normal counterpart [(Oh43+ × B37+) × C103+] were surface-sterilized by treatment with alcohol (95%, 1 min), and with bleach (10% Clorox, 3 min), followed by rinsing twice with sterile deionized water. Germination was carried out in the dark at 28° C by soaking and incubating the seeds with minimal amounts of distilled water in covered petri dishes. Seedlings (about 10 g wet weight) were removed on subsequent days for analysis. Samples were divided into three parts for the determination of aspartokinase, free lysine, and total lysine, respectively. Aspartokinase was extracted and assayed according to the procedure of Bryan and coworkers (15) and aspartokinase activity was expressed according to Black and Wright (16). Total aspartokinase units by these determinations were further converted to and expressed on a per seedling basis. The remainder of the seedling sample was oven-dried (65° C, 2 days), weighed, and ground. The ground sample was analyzed for total nitrogen by the micro-Kjeldahl procedure, and for total lysine

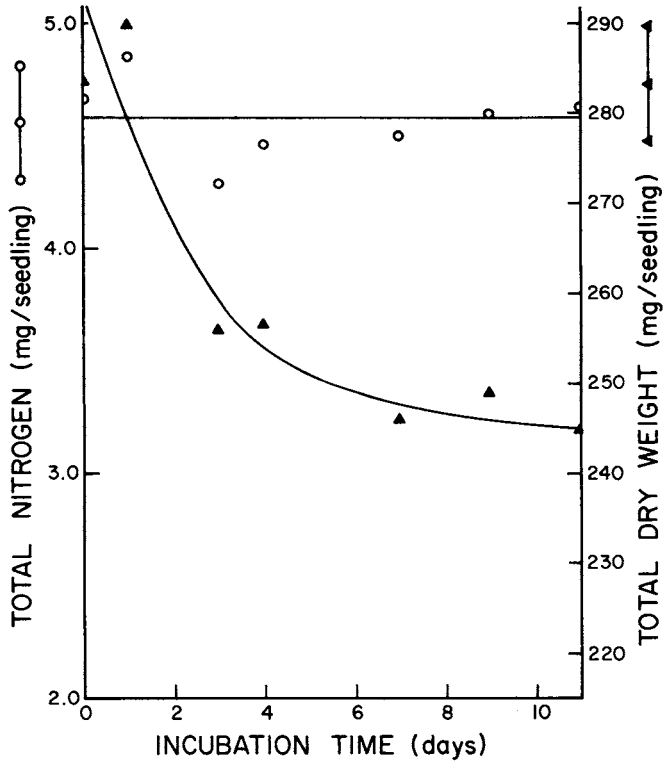

Fig. 1. Total dry weight and nitrogen content of normal maize seedlings during germination.

by acid hydrolysis, followed by ion-exchange chromatography on an automated amino acid analyzer (Beckman). Free (soluble) lysine in the seedling sample was extracted into millimolar HCl (100 ml) in a Waring Blendor. The extract (50 ml) was washed with ether (3 × 50 ml), and subjected to lysine determination by ion-exchange chromatography on the amino acid analyzer.

RESULTS AND DISCUSSION

The changes in total nitrogen and total dry weight of germinating normal and *opaque*-2 maize seedlings are shown in Figs. 1 and 2. The consistency in total nitrogen reflects the absence of an exogenous nitrogen source. There was a decline in total dry weight by about 15% in both instances.

The changes in total and free lysine content during germination are shown in Figs. 3 and 4. There are striking differences in the behavior of normal and *opaque*-2 maize. While normal maize seedlings show a net increase in their content of lysine (from 733 to 808 μg/seedling), there is a marked decrease (from 1160 to 880 μg/seedling) in the total lysine content in their *opaque*-2 counterparts. A similar observation concerning normal maize has been made by

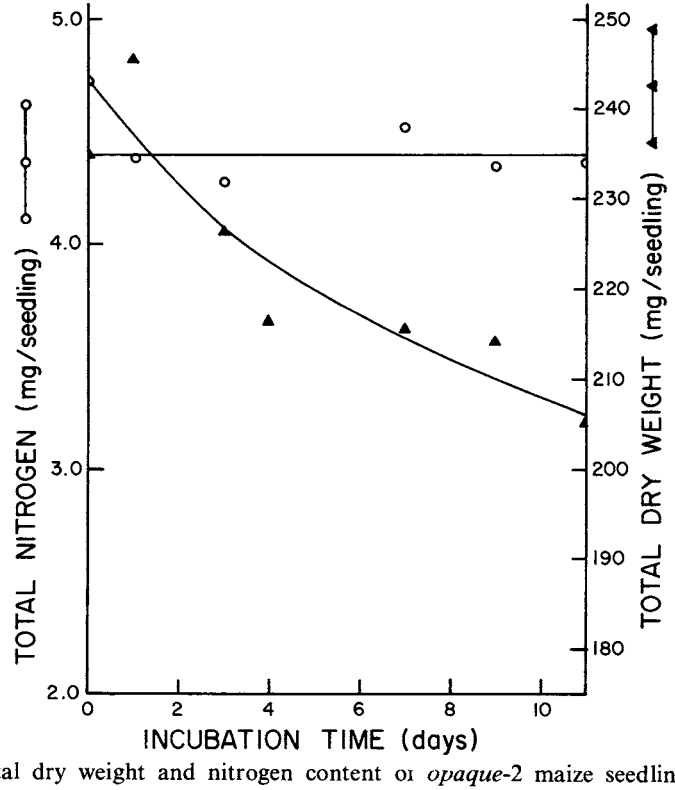

Fig. 2. Total dry weight and nitrogen content of *opaque*-2 maize seedlings during germination.

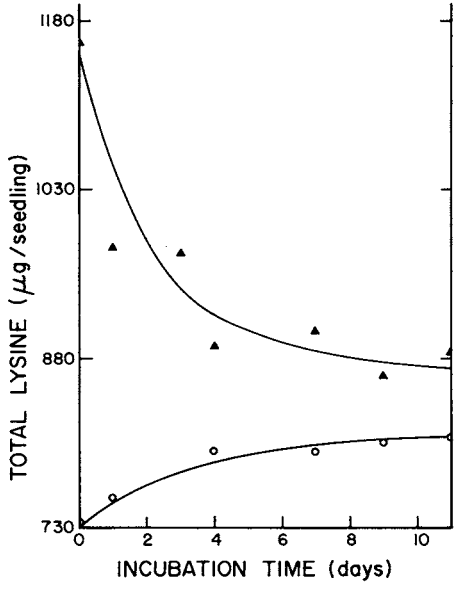

Fig. 3. Total lysine content of normal (0 - 0 - 0) and *opaque*-2 (▲ - ▲ - ▲) maize seedlings during germination.

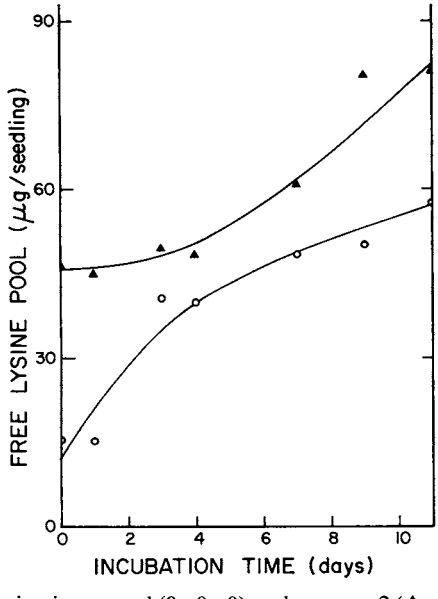

Fig. 4. Levels of free lysine in normal (0 - 0 - 0) and *opaque*-2 (▲ - ▲ - ▲) maize seedlings during germination.

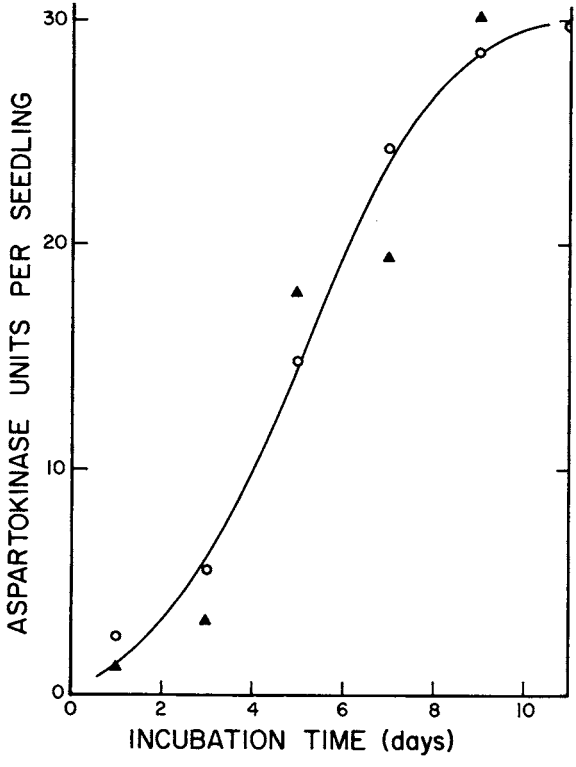

Fig. 5. Total aspartokinase activity in concentrated extracts from germinating normal (▲ - ▲ - ▲) and *opaque*-2 (0 - 0 - 0) seedlings over an 11-day period.

TABLE I
Aspartokinase Activity from Extracts of Germinating Normal and *Opaque*-2

Sample	Days from Germination	Seedlings: Aspartokinase activity (units/seedling)		
		Crude extract	Dialyzed extract	Concentrated extract[a]
Normal	3	0	3.5	3.3
Opaque-2	3	0	5.0	5.6
Normal	5	4.5	19.0	17.9
Opaque-2	5	0	15.2	14.85
Normal	7	5.4	21.1	19.4
Opaque-2	7	2.5	26.3	24.3
Normal	9	5.6	32.0	30.7
Opaque-2	9	3.4	33.2	28.6

[a]Proteins precipitated from the dialyzed extract at 60% ammonium sulfate saturation.

Tsai *et al.* (17). Further examination of these results shows that in normal maize the net increase in lysine content closely parallels the increase in the free lysine pool. The net increase in the levels of free lysine in the normal seedlings can be attributed to *de novo* synthesis. In the *opaque*-2 seedlings, however, the decrease in total lysine content is much more rapid than the corresponding increase in the levels of free lysine. These results suggest that during germination of *opaque*-2 maize, the catabolism of free lysine occurs rapidly, though it is generated at an even faster rate by the breakdown of storage proteins.

Lysine synthesizing ability of the seedlings was determined by measuring total levels of aspartokinase. The results are shown in Fig. 5 and in Table I. Figure 5 shows the total units of enzyme activity present, per seedling, in crude extracts from normal and *opaque*-2 maize. Table I lists the actual amounts of activity observed during the isolation procedures. It is evident from the results in Table I that, though no aspartokinase activity is present in the primary extracts from *opaque*-2 seedlings, the dialyzed extracts show activity equivalent to that of their normal counterpart, the activity of which is also enhanced upon dialysis. This observation is consistent with, and strongly indicates, feedback inhibition of aspartokinase activity by free lysine as observed earlier (15).

The observation that aspartokinase activity is generated at a similar rate during germination of normal and high-lysine maize signifies that the *opaque*-2 gene does not interfere significantly with the biosynthetic pathway for lysine. Feedback resistant mutant(s) have classically been defined as ones in which there is an excessive buildup of the effector metabolite (18). However, this is not strictly true in *opaque*-2 maize, for although there is a large increase in free lysine levels, they never become significantly higher than those of the other free amino acids[3]. Finally, production of certain forms of aspartokinase in microorganisms is known to be under genetic control via induction and repression mediated by lysine and threonine (14). The experiments described here do not rule out the possibility that, provided a similar situation exists in eukaryote systems, production of these enzyme forms may be free of repressive control by lysine and/or threonine in the high-lysine mutants of maize. However, any such genetic alteration would be subject to metabolic correction via feedback control of the enzymatic activity. In conclusion, it appears that the biosynthesis, utilization, and distribution of lysine during germination of normal and high-lysine maize are primarily governed by metabolic factors determining supply and demand at the cellular level. A further understanding of the biochemical nature of this mutation might best be sought through studies of the developing seed.

[3]Mertz, E. T. Unpublished observations.

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