CHANGES IN LYSINE AND TRYPTOPHAN CONTENT DURING GERMINATION OF NORMAL AND MUTANT MAIZE SEED[1]

R. A. JONES[2] and C. Y. TSAI, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

ABSTRACT

Cereal Chem. 54(3): 565-571

During germination, lysine and tryptophan contents increase in the embryo of normal maize such that by 4 days' germination, the total levels of these amino acids are comparable to those found in mature seed of the 'high-lysine' mutants, *opaque*-2 and *floury*-2. Germination of the mutants *opaque*-2, *floury*-2, or the double gene combination *opaque*-2 *floury*-2, on the other hand, does not result in a comparable enhancement in total levels of lysine and tryptophan. The results are discussed in relation to the phenotypic changes elicited by these mutants.

The primary role of the storage proteins in maize endosperm appears to be that of a repository of nitrogen, which benefits the embryo at the time of germination. Zein, the major storage protein in maize endosperm, is devoid of the essential amino acids lysine and tryptophan (1), and is therefore of poor nutritional quality for monogastric animals.

We have previously observed a correlation between the levels of lysine and tryptophan and the growth during germination of normal maize seed (2). In somewhat similar fashion, other workers have noted increases in lysine and tryptophan content in barley (3), and increases in free lysine levels in peas (4) accompanying germination. During this germination period, storage protein content markedly decreases (2,5). This suggests that zein may supply precursors required for the synthesis of these essential amino acids. In support of this, we now report that mutants containing reduced levels of zein do not demonstrate the dramatic increase in lysine and tryptophan content that normally accompanies germination.

RESULTS AND DISCUSSION

In the four genotypes examined, negligible growth of the root or shoot was observed during the initial 24-hr period of germination (Fig. 1). Following this initial period, *normal* (*N*) and *floury*-2 (fl_2) roots grew most rapidly with comparable kinetics. The growth of *opaque*-2 (o_2) roots, on the other hand, is slower, while growth of *opaque*-2 *floury*-2 (o_2fl_2) is intermediate. The induction of primary root growth in o_2 and o_2fl_2 occurs approximately 24 hr after that observed for *N* and fl_2. In all four genotypes, shoot growth is initiated about 24 hr after emergence of the primary root. Early shoot growth in o_2 and o_2fl_2, in contrast to *N* and fl_2, is slightly depressed.

To clarify the effect of the o_2 gene on primary root growth, seeds from several inbred lines homozygous for the o_2 gene and their normal counterparts were germinated (separately) as described in **Materials and Methods**. At appropriate intervals, the length (mm) of the primary root from 25 kernels was measured. All

[1]Journal Paper No. 6376. Purdue University Agricultural Experiment Station. This research was supported in part by a grant from the Lilly Endowment.

[2]Present address: Department of Vegetable Crops, University of California, Davis, CA 95616.

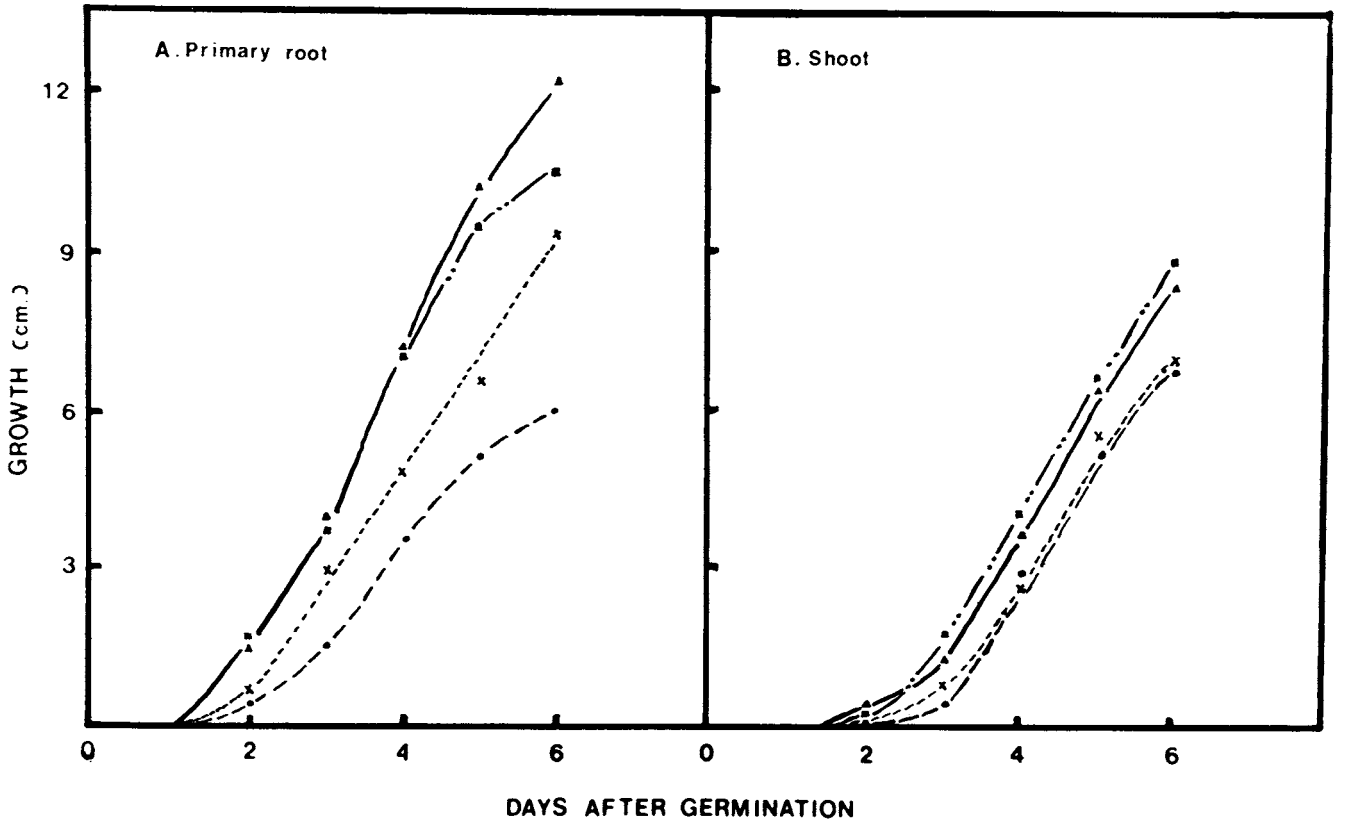

Fig. 1. Comparison of the growth of the primary root (A) and shoot (B) in four maize genotypes during germination of the inbred line W64A. (▲) *normal;* (●) *opaque*-2; (■) *floury*-2; (×) *opaque*-2 *floury*-2.

inbred lines tested (W64A, A545, C123, W22, B37, and B14) indicate that the presence of the o_2 gene results in a marked depression of growth (data not shown). Genetic alteration of the endosperm nitrogen reserves may therefore have adverse effects on the resultant growth metabolism of the embryo.

Presented in Table I are changes in reducing sugars, sucrose, total ethanol-soluble carbohydrate, and starch content at selected time course intervals. The reducing sugars, sucrose, and soluble carbohydrate contents in all four maize types show a similar pattern of an early decrease or little change in the levels during the first 36 hr, followed by a period of dramatic increase in levels over the remaining germination period. The rates of accumulation are significantly greater for the normal than for the three mutant-type seeds.

In 5.5 days, starch content is reduced 42% in *N* (Table I). Over a comparable period of time, the o_2, fl_2, and o_2fl_2 mutants show a decrease in starch content of 50, 60, and 53%, respectively. As the amount of starch decreases in *N*, the amount of soluble carbohydrate increases, such that the amount of digestible carbohydrate diminishes only slightly (about 25%).

Accompanying the 6-day period of germination, the total levels of lysine (Fig. 2) and tryptophan (Fig. 3) increase rapidly in *N* kernels. However, the increase of these two amino acids in the o_2 mutant was minimal (Figs. 2, B, and 3, B). Both fl_2 (Figs. 2, C, and 3, C) and o_2fl_2 (Figs. 2, D, and 3, D) show a small increase in lysine (18 and 20%, respectively) and in tryptophan. The changes observed in *N* maize are such that by 4 days, the apparent content of lysine (720 mg) and tryptophan (166 mg) per kernel approximates that found in mature seed carrying the mutant genes o_2 (1083 mg:216 mg) and fl_2 (833 mg:188 mg). Seed germination may therefore provide a practical and important means of

TABLE I
Changes in Carbohydrate Levels during Seed Germination of Normal and Mutant Maize Varieties (mg/kernel)

		Genotype				
	hr	+	o_2	fl_2	o_2fl_2	SE[a]
Starch	0	116.1	80.7	88.1	115.4	0.18
	36	08.0	75.9	85.6	97.3	0.13
	84	96.7	63.6	50.4	89.6	0.11
	132	70.5	42.7	34.5	53.7	0.10
Soluble sugars	0	5.71	7.20	4.82	6.94	0.13
	36	2.72	3.20	3.40	3.20	0.09
	84	15.5	9.9	11.6	13.8	0.11
	132	27.9	13.8	17.1	15.4	0.16
Reducing sugars	0	0.31	0.41	0.25	0.60	0.01
	36	1.01	1.13	0.37	1.12	0.06
	84	9.14	5.25	6.85	7.68	0.11
	132	13.64	8.63	9.30	8.90	0.10
Sucrose	0	3.20	4.62	3.35	3.84	0.06
	36	1.38	1.31	1.92	1.54	0.09
	84	5.29	3.85	3.43	4.07	0.11
	132	11.82	6.24	4.42	4.92	0.12

[a]Standard error of each mean value in column.

improving the nutritional value of corn protein.

Increases in lysine and tryptophan content in all four genotypes were observed in the embryo, while endosperm levels of these amino acids decreased simultaneously (Figs. 2, A–D, and 3, A–D). Since the levels of lysine and tryptophan increase in *N* embryos prior to detectable losses of these amino acids from the endosperm, it may be concluded that the increases are due at least partially to synthesis and not solely to utilization of pre-existing pools from

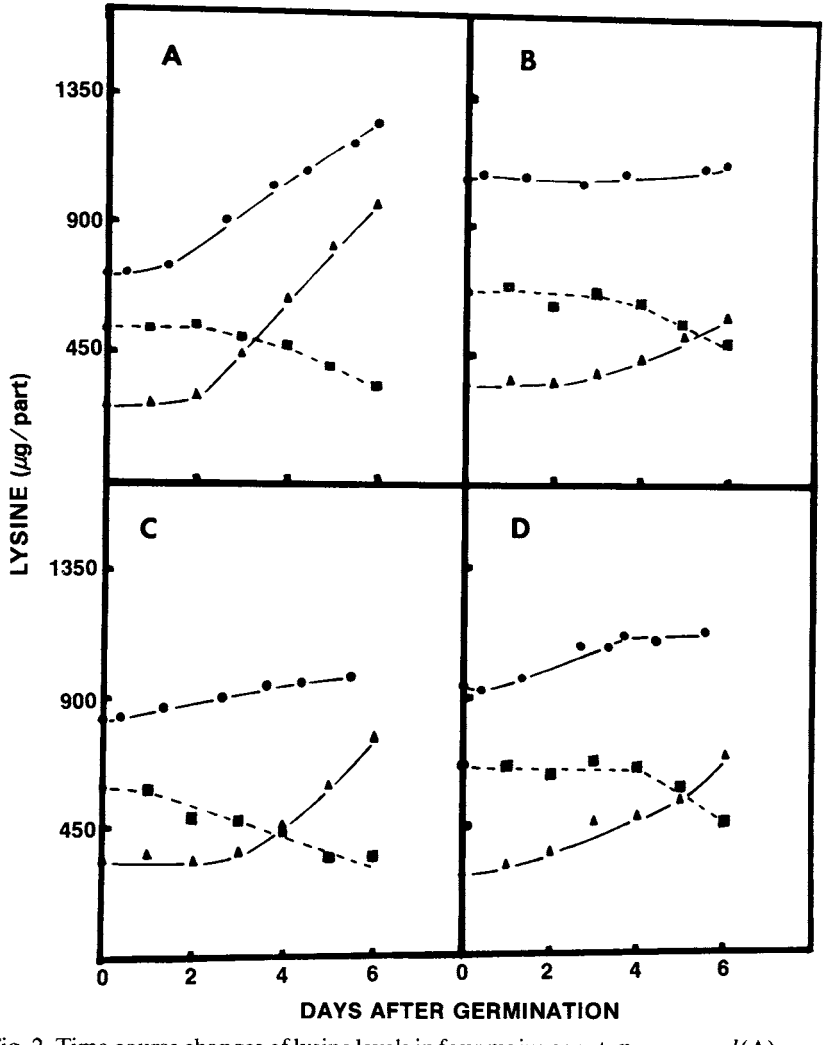

Fig. 2. Time course changes of lysine levels in four maize genotypes: *normal* (A), *opaque*-2 (B), *floury*-2 (C), and *opaque*-2 *floury*-2 (D). Seed of each type was germinated at 28° C in the dark. At appropriate intervals, seed was harvested and analyzed for lysine content in the whole seed (•), endosperm alone (■), and embryo alone (▲).

elsewhere in the seed. This increase is restricted to the embryo, suggesting that the proteins necessary for embryo growth and development require higher levels of lysine than do endosperm proteins. Previous workers have shown that such a difference exists in comparable proteins from barley (6).

Mature seeds of the mutant genotypes characteristically contain high levels of lysine and tryptophan (Figs. 2 and 3), and reduced zein content (7). An inverse relation between these two parameters exists in mature seed (8). The mutant seed's rate of lysine accumulation during germination is diminished significantly

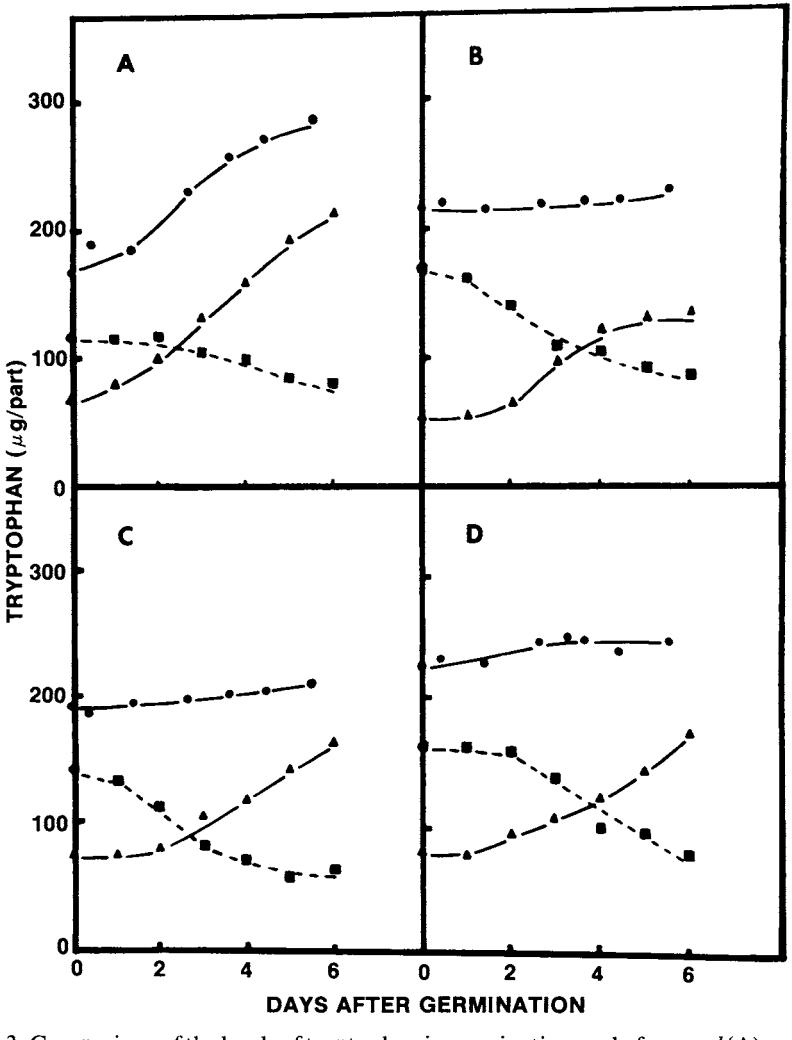

Fig. 3. Comparison of the levels of tryptophan in germinating seed of *normal* (A), *opaque*-2 (B), *floury*-2 (C), and *opaque*-2 *floury*-2 (D) genotypes. Seed of the various types was germinated at 28°C in the dark. (■) endosperm; (▲) embryo; (•) whole kernel.

(Fig. 2). In contrast, *N* maize seed, which contains low levels of lysine (Fig. 2) and high zein levels (2,8), increases dramatically in lysine content during germination. Inspection of Figs. 2 and 3 indicates the amino acid increase occurs earlier and at a faster rate in the *N* embryo than in the mutants. The kinetics of lysine loss from the endosperm in all four genotypes are similar, yet the kinetics of accumulation of lysine by the embryo differ. It is apparent that the two processes are not reciprocal, and it seems that amino acid synthesis, not merely transport, is required for increased lysine and tryptophan content of the embryo. The *de novo* synthesis of endosperm proteins requiring lysine may account for the retention of lysine within the endosperm. The correlation between the amino acid increases during germination and the initial zein content of normal and mutant varieties suggests that precursors necessary for lysine biosynthesis may be derived from zein. If this hypothesis is correct, the controlling factor in these increases may be the rate of mobilization of the zein reserves from the endosperm. The high-lysine varieties may supply the amino acid requirements for embryo growth directly and block further metabolism of zein. Evidence for this possibility will be reported in a separate communication.

MATERIALS AND METHODS

Plant Material

The maize (*Zea mays* L.) inbred W64A and its homozygous mutant versions *opaque*-2 (W64Ao_2), *floury*-2 (W64Afl_2), and the double mutant *opaque*-2 *floury*-2 (W64Ao_2fl_2) were grown during 1972 at the Purdue University Agronomy Farm, Lafayette, Ind.; self-pollinated W64Ao_2 was a spontaneous mutant, and W64Afl_2 and W64Ao_2fl_2 were nearly isogenic (all were recovered after six backcrosses to the recurrent parent) with the normal line. Ears were harvested at maturity and forced-air dried. Mature seed of the above genotypes was surface-sterilized in 10% Chlorox bleach for 5 min and subsequently rinsed with sterile distilled water. Twenty seeds with embryo side down were placed uniformly around the periphery of sterile petri dishes (9 cm) containing 0.9% agar. Plates were routinely incubated at 28° C in the dark. Material was harvested at appropriate intervals, rinsed briefly with sterile distilled water, frozen on Dry Ice, and stored at −20° C. Plates showing microbial contamination were discarded.

Seed Germination

Following the collection of a complete germination series, the material was lyophilized to constant weight, ground in a Waring Blendor, then reduced to a fine powder in a miniature ball-mill (Crescent Dental Manufacturing Co., Chicago, Ill.). This was the starting material for all biochemical determinations. For those experiments requiring the isolation of individual seed parts, the embryo was rapidly dissected from frozen endosperm before lyophilization.

Carbohydrate and Nitrogen Determinations

Duplicate aliquots of hot 80% ethanol extracts were evaporated to dryness in a cool air stream. The residues were resuspended in 1 ml of distilled water, and reducing sugars were determined by the Somogyi method (9), and sucrose by the Roe method (10). Total soluble carbohydrate was assayed by the Anthrone

method (11). The starch content of the germinating seeds was determined by a macro-method (12). Analyses were performed on duplicate 1-g samples.

Total nitrogen analyses were performed on duplicate 100-mg samples of the finely powdered seed preparations by a micro-Kjeldahl procedure (13). Lysine was determined by the method of Tsai *et al.* (14), and total tryptophan was assayed by the method of Dalby and Tsai (15).

Literature Cited

1. MOSSE, J. Alcohol soluble proteins of cereal grains. Fed. Proc. 25: 1663 (1966).
2. TSAI, C. Y., DALBY, A., and JONES, R. A. Lysine and tryptophan increases during germination of maize seed. Cereal Chem. 52: 356 (1975).
3. FOLKES, B. F., and YEMM, E. W. The respiration of barley plants X. Respiration and the metabolism of amino acids and protein in germinating grain. New Phytol. 57: 106 (1958).
4. LARSON, L. A., and BEEVERS, H. Amino acid metabolism in young pea seedlings. Plant Physiol. 40: 424 (1965).
5. INGLE, J., BEEVERS, L., and HAGEMAN, R. H. Metabolic changes associated with the germination of corn. I. Changes in weight and metabolites and their redistribution in the embryo axis, scutellum, and endosperm. Plant Physiol. 39: 735 (1964).
6. FOLKES, B. F., and YEMM, E. W. The amino acid content of the proteins of barley grains. Biochem. J. 62: 4 (1956).
7. TSAI, C. Y., and DALBY, A. Comparison of the effect of *Shrunken*-4, *Opaque*-2, *Opaque*-7, and *Floury*-2 genes on the zein content of maize during endosperm development. Cereal Chem. 51: 825 (1974).
8. DALBY, A. Rapid method for determining the zein content of whole maize seed or isolated endosperm. Cereal Chem. 51: 586 (1974).
9. NELSON, N. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153: 375 (1944).
10. ROE, J. H. A colorimetric method for the determination of fructose in blood and urine. J. Biol. Chem. 107: 15 (1934).
11. ROE, J. H. The determination of sugar in blood and spinal fluid with anthrone reagent. J. Biol. Chem. 212: 335 (1955).
12. McGUIRE, J. P., and ERLANDER, S. R. Quantitative isolation and dispersion of starch from corn kernels without degradation. Staerke 18: 342 (1966).
13. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Official methods of analysis (11th ed.), p. 858. The Association: Washington, D.C. (1970).
14. TSAI, C. Y., HANSEL, L. W., and NELSON, O. E. A colorimetric method of screening maize seed for lysine content. Cereal Chem. 49: 572 (1972).
15. DALBY, A., and TSAI, C. Y. Acetic anhydride requirement in the colorimetric determination of tryptophan. Anal. Biochem. 63: 283 (1975).

[Received August 13, 1976. Accepted October 28, 1976]