LYSINE AND TRYPTOPHAN INCREASES DURING GERMINATION OF MAIZE SEED[1]

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ABSTRACT

During the germination of normal maize, increases in the concentration of lysine and tryptophan and decreases in the protein zein occur. Germination may thus offer a method for converting nutritionally poor-quality plant protein to a higher quality for human and animal use.

The storage proteins of maize seed are of poor nutritional quality for humans and monogastric animals. This is largely because of the low levels of lysine and tryptophan in the endosperm. The low level of lysine is the result of preponderant

[1]Journal Paper No. 5523, Purdue University Agricultural Experiment Station. This research was supported in part by a grant from the Lilly Endowment.

amounts of the lysine-poor prolamine, zein, which may account for as much as 50 to 60% of the total endosperm nitrogen in normal corn varieties.

To date, the most successful approach to improving the nutritional value of maize endosperm proteins, other than by direct supplementation, has been through the discovery that several mutant genes increase endosperm lysine and tryptophan levels (1,2,3). Similar genes have now been found in barley (4,5) and sorghum (6).

An alternative scheme for improving the nutritional value of corn, which ought to be readily applicable to existing lines and hybrids with their known and desirable characters, would be to permit the biochemical processes occurring during germination to mobilize the zein and produce the nutritionally high-quality proteins the plant needs for its own growth and development. The following results demonstrate that this may well be a valid and valuable approach to increasing the world supply of high-quality protein. It should be noted that although we were seeking simplicity of technique, we employed agar plates, seed surface sterilization, and asepsis in order to provide reliable experimental results unconfounded by microbial contamination.

MATERIALS AND METHODS

Seeds of the maize inbred W64A, homozygous normal (+/+), were surface-sterilized by treatment with 70% ethanol for 1 min, 10% Clorox® bleach for 5 min, followed by three rinses in sterile, deionized water. Batches of 25 seeds were then placed on 1% agar in 9-cm petri dishes, embryo down and seed base toward the center of the plate. The plates were incubated at 28° C in the dark, but without regard to occasional brief exposure to light at sampling times. The material was harvested at 24-hr intervals, washed to remove traces of agar, frozen in Dry Ice, and lyophilized. The dried material was reduced to a fine powder for total nitrogen (7), lysine (8), tryptophan (9), zein (10), reducing sugar (11), sucrose (12), and starch (13) analyses by grinding in a Waring Blendor followed by a Wig-L-Bug (Crescent Dental Mfg. Co., Chicago). When necessary, the embryo and endosperm were separated by dissection immediately after harvesting from agar. For comparison, analyses of ungerminated seed of the same inbred, homozygous for the 'high-lysine' mutant genes *opaque*-2 (W64Ao_2) and *floury*-2 (W64Afl_2), were also performed. Data in Fig. 1 are expressed per kernel since this permits comparison of the various parameters on the unchanging basis of the biological unit. The tables show data on a unit weight basis, which is more familiar to the nutritionist. The data are reproducible, similar results having been obtained in seven different experiments.

RESULTS AND DISCUSSION

It may be seen in Fig. 1 (A to F) that, accompanying the shift in dry matter (A) and total nitrogen (C) from endosperm to embryo during germination, there is an apparently large and rapid increase in total lysine (E) and tryptophan (F) and a corresponding decline in zein (D). These changes are such as to yield, within 2 to 3 days, apparent levels of lysine and tryptophan per kernel equivalent to those found in mature seeds carrying the mutant genes o_2 and fl_2. The increases of lysine and tryptophan are both confined to the embryo (E and F).

On a unit-weight basis these changes take somewhat longer to reach equivalence with the mutant gene levels (Table IA). This is because of the lower per-kernel dry weights of the mutant seed (Fig. 1A).

During germination, the amount of starch decreases about 50% in 5 days. Meanwhile reducing sugars and sucrose increase about 60-fold and 3-fold, respectively. Thus, the total digestible carbohydrate (as measured) decreases by only about 30% (Table IA).

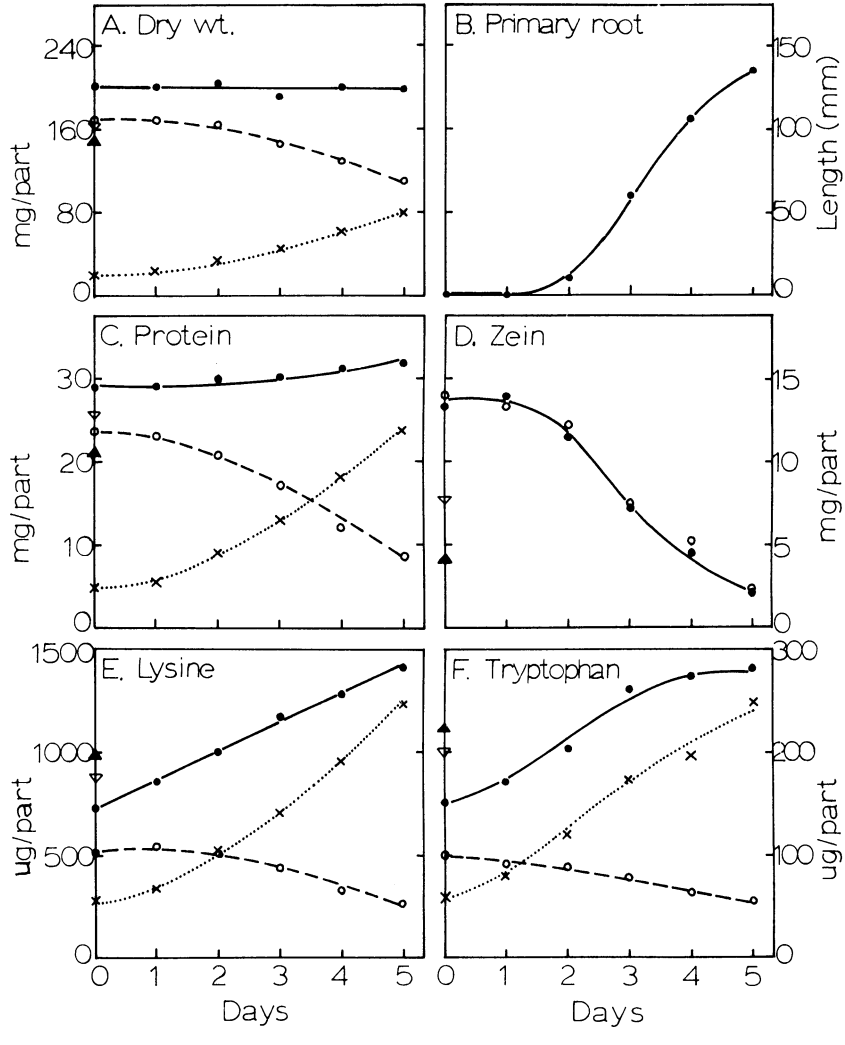

Fig. 1. Changes in dry weight (A), length of primary root (B), protein (C), zein (D), lysine (E), and tryptophan (F) during germination of maize inbred W64A at 28° C. (•) Normal whole kernel; (O) normal endosperm; (×) normal embryo; (▲) *opaque*-2 whole kernel; (▽) *floury*-2 whole kernel.

To further test this method of altering the lysine, tryptophan, and zein levels of corn in a nutritionally favorable direction, a commercial hybrid (WF9 × M14) was examined. Approximately 300 unsterilized seeds were distributed over moist paper towels in a 22 × 33-cm Pyrex ® baking dish, covered with Saran® wrap in which 16 small pinholes were punched, and incubated at 30°C. The material was harvested and treated as described under **Methods**. There was no visible fungal contamination after 5 days despite the lack of sterile precautions in this instance. The analyses (Table IB) indicate the apparent applicability of the method in this case also in terms of the increase in lysine and tryptophan, and the decrease in zein. Similarly other parameters behave as observed previously.

In an attempt to confirm the changes observed in lysine values in W64A+ by an independent method, samples of 5-day germinated seed were assayed by ion-exchange chromatography. A consistent increase in lysine of more than 50% over the ungerminated control was reported in separate analyses performed by three different laboratories[2]. This places the germinated lysine value intermediate between the ungerminated normal and o_2 values and, therefore, lower than the value obtained colorimetrically. Although the reasons for this discrepancy have not yet been resolved, both methods of analysis concur in demonstrating an appreciable increase in lysine as a result of germination and encourage hope for the validity of the approach.

It is still necessary to demonstrate by feeding trials that the higher levels of lysine and tryptophan accompanying germination do lead to improved nutritive value. If this test is positive, then a method for converting a readily available, but

[2]The laboratories of E. T. Mertz, J. D. Axtell, and Shuman Chemical Laboratories, Battle Ground, Ind.

TABLE I
Changes (per unit weight) in Protein, Zein, Lysine, Tryptophan, Reducing Sugars, and Starch during Germination

Sample	Days after Germination	Total Protein %	Zein %	Lysine %	Tryptophan %	Reducing Sugars %	Sucrose %	Starch %
A. Comparison between the germinating normal maize inbred W64A and mature seed of its homozygous high-lysine mutants *opaque*-2 and *floury*-2								
W64Ao_2	0	13.5	2.6	0.64	0.14	0.15	2.82	61.8
W64Afl_2	0	15.3	4.8	0.55	0.12	0.12	2.67	61.0
W64A+	0	14.4	7.0	0.38	0.08	0.14	1.87	61.0
W64A+	1	14.5	6.9	0.43	0.09	0.35	1.00	60.9
W64A+	2	14.7	5.5	0.48	0.11	1.95	1.42	58.1
W64A+	3	15.5	3.8	0.60	0.14	4.20	2.65	51.7
W64A+	4	15.6	2.3	0.64	0.14	7.25	4.50	41.0
W64A+	5	16.0	1.1	0.71	0.14	9.00	5.10	30.0
B. Germinating maize hybrid WF9 × M14								
WF9×M14	0	12.2	4.6	0.38	0.08	0.37	2.32	65.3
WF9×M14	2	12.1	3.2	0.43	0.09	2.00	2.00	60.0
WF9×M14	3	12.5	1.9	0.50	0.10	5.70	4.05	50.1
WF9×M14	4	12.2	1.1	0.61	0.13	10.75	5.75	38.4
WF9×M14	5	12.1	0.6	0.72	0.13	14.50	7.80	28.3

nutritionally poor, plant protein into a protein source of high quality becomes available. At the very least this may be of value for animal feed. The possibility of applying the same technique to other cereal grains also merits attention, since increases in lysine and tryptophan in germinating barley have been reported (14). Differences between sorghum grain and malt are reported to be small in this respect, however (15).

Acknowledgments

We thank E. T. Mertz and J. D. Axtell for ion-exchange lysine determinations.

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[Received August 29, 1974. Accepted September 27, 1974]