Phytochemistry, Vol. 24, No. 2, pp. 225–229, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

EFFECT OF SUCROSE ACCUMULATION ON ZEIN SYNTHESIS IN MAIZE STARCH-DEFICIENT MUTANTS*

LISA LEE† and CHARLES Y. TSAI‡

†Department of Biological Sciences and ‡Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, U.S.A.

(*Received* 11 *May* 1984)

Key Word Index—*Zea mays*; Gramineae; maize; zein synthesis; regulation; sucrose accumulation.

Abstract—Starch-deficient maize (*Zea mays*) mutants, *brittle*-2 (*bt*2), *brittle*-1 (*bt*), and *shrunken*-2 (*sh*2), which accumulated large quantities of sucrose, had less than normal amounts of zein (the major storage protein) in the endosperm. Reduction of zein synthesis in the starch-deficient mutants was negatively correlated with the accumulation of sucrose and low osmotic potential in the developing endosperms. When radioactive amino acids were injected into the shank below ears that segregated for the starch-deficient mutant and normal kernels at 28 days post-pollination, mutant kernels absorbed only *ca* 22–36% of the labelled amino acids found in their normal controls. Thus, a low osmotic potential in the mutant endosperm may favour water movement but reduce solute movement. The inability of amino acids to move into the mutant endosperms, therefore, in part explains the reduction of zein accumulation in starch-deficient mutant endosperms.

INTRODUCTION

Maize starch-deficient mutants, such as *brittle*-1 (*bt*), *brittle*-2 (*bt*2) and *shrunken*-2 (*sh*2), have low starch content and high sucrose accumulation in their endosperms [1]. The reduction of starch synthesis in *sh*2 or *bt*2 mutant endosperm is due to the deficiency of ADPglucose pyrophosphorylase activity [2–5]. In addition to the effect on starch synthesis, these starch-deficient mutants, like *opaque*-2 (*o*2) mutants, also reduce zein (major storage protein) accumulation [6–9]. However, the factors affecting zein synthesis in these mutant endosperms remain unknown.

Because zein synthesis responds dynamically to nitrogen treatment [10, 11], the reduction of zein synthesis in starch-deficient mutants may result from the lack of substrate availability. To test this hypothesis, maize ears which segregated for normal and starch-deficient mutant kernels in a one-to-one ratio were used to study the movement of radioactive amino acids into normal and mutant kernels.

RESULTS

Fresh weight, dry weight and water content in developing endosperms

The *bt*2 endosperm had the highest water content (Fig. 1A) and the greatest fresh weight (Fig. 1B) but lowest dry weight of the three genotypes (*bt*2, *o*2 and normal) studied, and the dry weight ceased to increase after 30 days post-pollination (Fig. 1C). The rate of dry matter accumulation in *o*2 endosperm was slightly lower than normal, and reached maximum dry weight *ca* 40 days after pollination. This observation agrees with previous reports [6, 12]. However, water content in the *o*2 endosperm was higher than that of the normal genotype throughout development (Fig. 1A).

Sucrose, free amino acid and reducing sugar concentrations in developing endosperms

The sucrose concentration of *bt*2 endosperm was more than two-fold higher than that of the normal genotype from 12 days after pollination until 37 days after pollination (Fig. 2A). Although the sucrose concentration of the *o*2 endosperm was *ca* 90% of that of normal endosperm during development, the free amino acid concentration of the *o*2 endosperm was the highest among all the genotypes studied (fig. 2B). The *bt*2 mutant and the normal genotype contained similar amounts of free amino acids. Reducing sugars in all genotypes were highest at 10 days after pollination and decreased similarly throughout endosperm development (Fig. 2C).

At 28 days post-pollination, sucrose accounted for *ca* 45% of the total osmotica (sum of the three osmotica, i.e. sucrose, free amino acids and reducing sugars) in the normal genotype; however, sucrose constituted more than 60% of its total osmotica in the *bt*2 endosperm. Thus, the *bt*2 endosperm had a total osmotica *ca* 1.5-fold higher than that of the normal genotype. The osmotica of the *o*2 endosperm was *ca* 1.3 times higher than that of the normal genotype at 28 days after pollination.

Zein content in developing endosperms

Zein synthesis in the normal genotype started rapidly at 12 days after pollination and continued to increase until 50 days after pollination. The *o*2 endosperm, on the other hand, did not start to accumulate zein protein until 16

*Supported in part by a grant from NIH Grant GM 25045. Journal Paper No. 9685. Purdue University Agricultural Experiment Station.

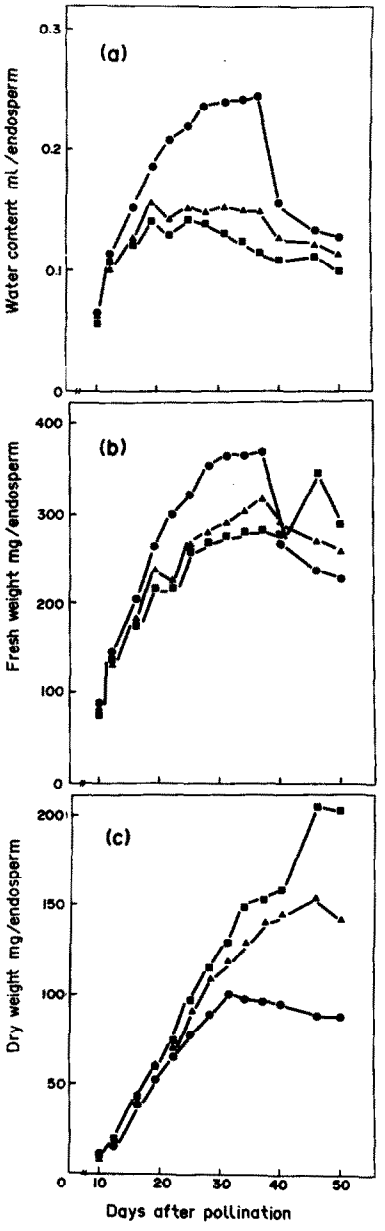

Fig. 1. Water contents (A), fresh weights (B), and dry weights (C) of normal (■), *o2* (▲) and *bt2* (●) endosperms during development.

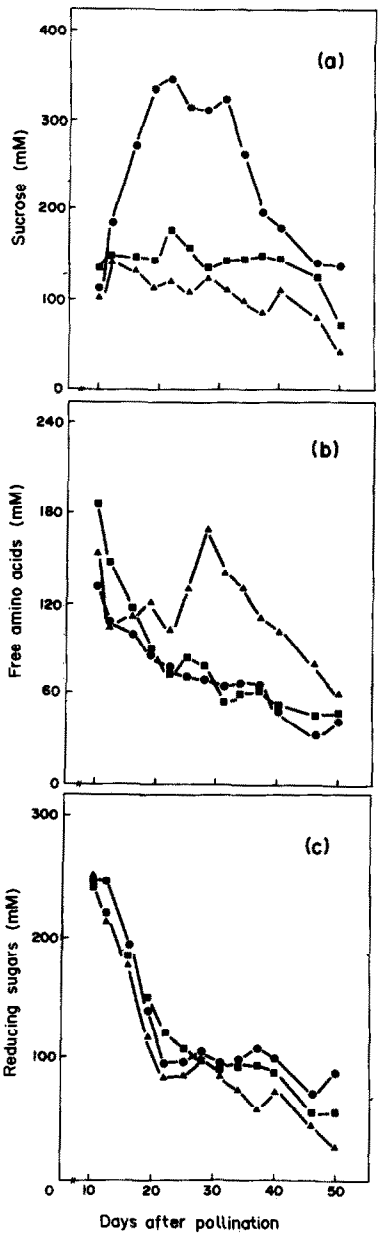

Fig. 2. Concentrations of sucrose (A), free amino acids (B) and reducing sugars (C) of normal (■), *o2* (▲) and *bt2* (●) endosperms during development.

days after pollination, and terminated zein synthesis at *ca* 28 days post-pollination. These results agree with a previous observation [11]. Although zein synthesis in the *bt2* endosperm started 12 days after pollination, it proceeded at a lower rate than did synthesis in the normal genotype. In the *bt2*, zein protein accumulation peaked at around 34 days after pollination (Fig. 3). At maturity, *bt2* and *o2* mutants contained 40% and 35% of normal zein content, respectively.

Sucrose accumulation and zein synthesis

At 22 days post-pollination, the sucrose concentrations in the 13 genotypes studied ranged from 115 to 493 mM (Table 1), and zein content correlated negatively with sucrose concentration ($r = -0.57$).

Absorption of radioactive amino acids, osmotica level and osmotic potential of the segregating normal and mutant kernels

Like *bt2* mutants, *sh2* and *bt* mutants also exhibited higher sucrose levels than their normal counterparts (Table 2). At 28 days post-pollination, the *sh2* kernels and the *bt* kernels contained sucrose levels *ca* 3-fold and 1.5-fold higher than their normal counterparts, respectively. The high sucrose content in *sh2* kernels contributed *ca* 80% of the total osmotica and *sh2* kernels had 2.3-fold

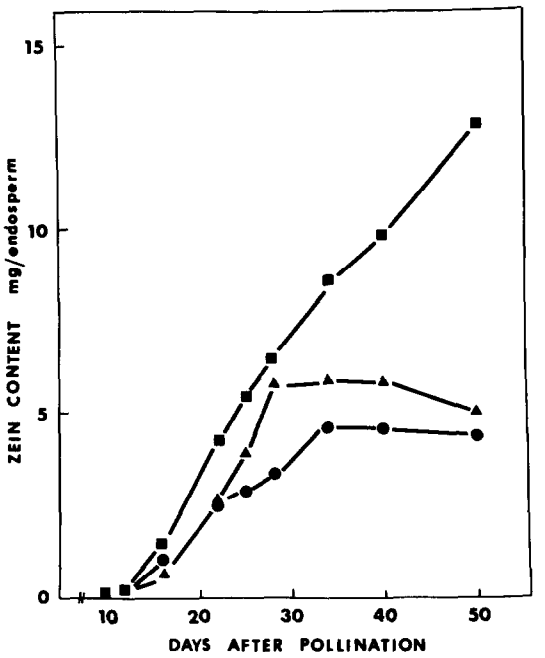

Fig. 3. Zein contents of normal (■), *o2* (▲) and *bt2* (●) endosperms during development.

Table 1. Sucrose concentration and zein content of 22-day-old maize endosperms

Genotype	Sucrose* (mM)	Zein* (mg/endosperm)
Normal	177 ± 5	4.30 ± 0.1
o2	115 ± 0	2.69 ± 0
bt2	339 ± 6	2.67 ± 0.2
bt2;o2	399 ± 14	0.47 ± 0
bt	248 ± 0	3.38 ± 0
bt;o2	493 ± 4	0.34 ± 0
sh2	320 ± 0	2.33 ± 0.1
su	247 ± 4	2.50 ± 0.1
su;o2	269 ± 2	0.39 ± 0
su2;o2	130 ± 2	1.48 ± 0
du	182 ± 0	3.13 ± 0.1
du;o2	135 ± 2	1.08 ± 0
wx;o2	145 ± 3	1.89 ± 0

*Means of two measurements ± s.e.

higher osmotica than normal kernels. The osmotic potential of the *sh2* kernel was −14 bars as compared to −6.6 bars in the normal control. The *bt* kernel had −12.6 bars vs −8.6 bars osmotic potential in control kernels from the same ear. These data agreed with the results obtained from estimation by the Van't Hoff's equation (Table 2). This low osmotic potential resulted in a plumpness of mutant kernels. At this stage of development, *sh2* kernels absorbed 24% of [^{14}C]leucine and 22% of [^{14}C]glutamine found in the segregating normal controls, while *bt* kernels contained *ca* 31% of [^{14}C]leucine and 36% of [^{14}C]glutamine found in the normal (Table 3).

DISCUSSION

The synthesis of zein in the maize endosperm is not only subjected to genetic control, but also responds dynamically to nitrogen treatment. Presumably, as more amino acids move into the endosperm, more zein is synthesized while non-zein protein production is little affected [10, 11]. Thus, a restricted amino acid movement into kernels could explain, in part, the decrease of zein synthesis in the starch-deficient mutants.

Starch-deficient mutants accumulate large quantities of sucrose in their endosperms. Accordingly, the large amounts of sucrose in the mutant endosperms generate a much more negative osmotic potential as compared to their normal counterparts. A low osmotic potential condition reduced transport of solute, but favoured movement of water into kernels as indicated by increased water content (Table 2), plumpness in kernel appearance and decreased radioactive amino acid absorption in the mutant kernels (Table 3). Thus, a reduced zein synthesis in the developing endosperms of all starch-forming mutants was negatively correlated ($r = -0.57$) with sucrose concentrations (Table 1).

Among the three genotypes studied, i.e. *bt*, *sh2* and normal, the *sh2* mutant accumulated the largest quantities of sucrose in the endosperm, generated the lowest osmotic potential (−14 bars), and had the least amount of radioactive amino acid incorporated into the kernel (*ca* 25% of the normal control). Accordingly, this mutant produced the smallest amount of zein. The *bt* mutant, on the other hand, accumulated an amount of sucrose intermediate between *sh2* and normal, and had intermediate osmotic potential, amino acid incorporation, and zein synthesis in the endosperm. These observations clearly support the notion that the accumulation of sucrose in the endosperm generates a low osmotic potential to reduce amino acid movement, and hence a reduction in zein synthesis. Although the reduction of zein synthesis in starch-deficient mutants may be attributable to the lack of substrate availability, a high RNase activity in their endosperms may also play a role in reducing zein content. High sucrose concentrations in the starch-deficient mutants 'induce' a high RNase activity in their endosperms [13]. Because the bulk of zein, unlike albumins, is synthesized at the later stage of endosperm development [14] and coincided with increases in RNase activity [15], the high RNase activity may reduce zein synthesis in starch-deficient mutant by degrading rRNAs and zein mRNAs [15, 16].

The lower than normal sucrose concentration observed in the developing *o2* endosperms (Table 1, Fig. 2) was presumably the result of the accumulation of free amino acids [10]. Like the *sh2* and *bt* systems in which high sucrose concentrations generated a low osmotic potential to reduce amino acid movement into the kernel, the *o2* kernels also generated a low osmotic potential due to their amino acid accumulation; therefore, *o2* kernels incorporated significantly less sucrose than normal kernels in the same ear. Similar results were reported by Sodek and Wilson [17] in their studies of the incorporation of [^{14}C]leucine and [^{14}C]lysine into protein in normal and *o2* endosperm. Therefore, the movement of carbon and nitrogen into the kernel is interrelated, and is under the control of osmotic regulation.

EXPERIMENTAL

Collection of maize materials. Maize inbred Oh43 and its isogenic mutants, *o2* and *bt2*, were grown at the Purdue Agronomy Farm in 1979 and 1980. All plants were self-

Table 2. Water content, total osmotica and osmotic potential of normal and mutant kernels obtained from heterozygous ears at 28 days after pollination

	Normal	*sh2**	Normal	*bt*†
Water content (ml/kernel)	0.20 ± 0.01	0.36 ± 0.01	0.17 ± 0.01	0.29 ± 0.01
Fresh weight (mg/kernel)	366 ± 3	514 ± 5	308 ± 6	416 ± 6
Dry weight (mg/kernel)	140 ± 1	122 ± 1	124 ± 4	97 ± 4
Sucrose (mM)	143 ± 1	431 ± 7	159 ± 1	225 ± 2
Free amino acids (mM)	80 ± 1	78 ± 1	101 ± 1	80 ± 1
Reducing sugars (mM)	44 ± 1	54 ± 1	40 ± 1	45 ± 2
Total osmotica (mM)	267 ± 2	563 ± 9	300 ± 4	350 ± 4
Osmotic potential (bars)‡	−6.6 ± 2.6	−14.0 ± 2.1	−8.6 ± 0.6	−12.6 ± 2.9
Estimated osmotic potential (bars)§	−6.6 ± 0.1	−13.9 ± 0.2	−7.4 ± 0.1	−8.7 ± 0.1

Each value represents a mean of two measurements ± s.e. except the osmotic potential determination which is a mean of 14 measurements.

*In W64A × Illini super chief background.

†In W64A × W22*bt* background.

‡Determined by psychromatic method.

§Estimated by Van't Hoff's equation.

Table 3. Incorporation of radioactive amino acids into segregated starch-deficient mutant and normal control kernels at 28 days after pollination

	Radioactivity incorporated (cpm)*			
	Normal	*sh2*†	Normal	*bt*‡
[^{14}C]Leucine	1890 ± 510	444 ± 239	2180 ± 720	671 ± 407
[^{14}C]Glutamine	2180 ± 900	474 ± 333	2550 ± 450	914 ± 421

*Means of 15 measurements ± s.e.

†In W64A × Illini super chief background.

‡In W64A × W22*bt* background.

pollinated. Ear samples were harvested at 8–50 days after pollination. Ears were frozen in liquid N_2 immediately after harvest. Intact kernels were removed from cobs and stored at −20°. For each stage, at least 6 ears were harvested, and the kernels were pooled.

In order to correlate zein synthesis with sucrose accumulation in the kernel, ear samples of starch-forming mutants (in Oh43 background) that accumulate various amounts of sucrose were harvested at 22 days post-pollination. These isogenic single and double mutants included: *bt*, *sugary*-1 (*su*), *sugary*-2 (*su2*), *dull* (*du*), *waxy* (*wx*), *bt2*, *sh2*, *bt2;o2*, *bt;o2*, *su;o2*, *su2;o2*, *du;o2* and *wx;o2*.

Determination of water content. With the exception of 8-, 10- and 12-day materials, 50 endosperms from each developing stage were weighed before and after drying at 103° to determine the H_2O content and dry wt of the endosperm. For 8-, 10- and 12-day materials, whole kernels were used because of difficulties in separating embryos and endosperms.

Extraction and determination of zein proteins. Developing endosperm samples were lyophilized to constant wt and ground in a Waring blender. A 1 g sample was powdered in a miniature ball mill (Wig-L-Bug, Crescent Dental Mfg. Co., Chicago, IL) for 5 min, and defatted for 48 hr with hexanes in a Soxhlet apparatus. These defatted samples provided the starting materials for zein extraction [14].

Measurement of sucrose, free amino acid and reducing sugar concentrations. One part of frozen endosperms were homogenized in 3 parts (w/v) of 70% EtOH with a Polytron homogenizer. The homogenate was incubated at room temp. for 20 min before centrifugation at 17000 *g* for 15 min [18]. The pellet was extracted twice more using the same procedures, and the EtOH-soluble fractions were combined. Three vols. of H_2O were added to the EtOH-soluble fraction to precipitate the EtOH-soluble proteins, and the suspension was kept chilled for 1 hr before centrifugation at 17000 *g* for 15 min. The supernatant was used to determine the concns of sucrose [19], reducing sugars [20] and free amino acids [21]. Each of the osmotica (mg/endosperm) was divided by H_2O content and by its gram MW to estimate their concns (mM).

Measurement of osmotic potential. Osmotic potential of the kernels was measured by the vapour pressure method using a PS-625 psychrometer. The osmotic potential contributed from the major solutes was estimated by the Van't Hoff's equation [22].

Absorption of radioactive amino acids by segregating normal and mutant kernels. In a different experiment to measure the effect of sucrose accumulation on amino acid movement into the kernel, plants heterozygous for the *sh2* allele (W64A × Illini super chief), and the *bt* allele (W64A × W22*bt*) were grown at the Purdue Agronomy Farm in 1981. They were pollinated with the mutant pollen, W64A*sh2* or W64A*bt*, respectively, so that each ear contained normal and mutant kernels in a 1:1 ratio. Mutant kernels can be distinguished readily from normal kernels by their plump appearance or by testing for sucrose content. At 28 days after pollination, 5 μCi [^{14}C]leucine (50 mCi/mmol) or

[^{14}C]glutamine (50 mCi/mmol) was injected into the shank below the ear. Two plants were used for each study. Fifteen kernels each of normal and mutant were harvested from every segregating ear 4 hr after injection; at that time, *ca* 15% of the total radioactivity was incorporated into kernels. Mutant and normal kernels were harvested from the same part of the ear to avoid uneven distribution of nutrients. Radioactivity incorporated into the kernels was determined by crushing individual kernels in a scintillation vial, and incubating crushed kernels for 5 days at room temp. in Omnifluor (New England Nuclear) containing 3% (v/v) Protosol (New England Nuclear), then measuring radioactivity by liquid scintillation spectroscopy. Untreated normal and mutant kernels from the same stage of development were processed in a similar manner, and a known quantity of ^{14}C-labelled leucine was added to the vial to determine the percentage of quenching.

The *in vivo* concns of free amino acids, reducing sugars and sucrose, and osmotic potentials in the untreated kernels were also measured at the same stage of development.

Acknowledgement—We thank Dr. Ray A. Bressan of the Department of Horticulture, Purdue University for the use of the psychrometer.

REFERENCES

1. Creech, R. G. (1965) *Genetics* **51**, 1175.
2. Dickinson, D. B. and Preiss, J. (1969) *Plant Physiol.* **44**, 1058.
3. Hannah, L. C. and Nelson, O. E. (1976) *Biochem. Genet.* **14**, 547.
4. Tsai, C. Y. and Nelson, O. E. (1966) *Science* **151**, 341.
5. Weaver, S. H., Glover, D. V. and Tsai, C. Y. (1972) *Crop. Sci.* **12**, 510.
6. Dalby, A. and Tsai, C. Y. (1975) *Crop Sci.* **15**, 513.
7. Glover, D. V., Crane, P. L., Misra, P. S. and Mertz, E. T. (1975) in *High-Quality Protein Maize*, p. 228. Dowden, Hutchinson & Ross, Stroudsburg, PA.
8. Misra, P. S., Mertz, E. T. and Glover, D. V. (1975) *Cereal Chem.* **52**, 161.
9. Tsai, C. Y., Larkins, B. A. and Glover, D. V. (1978) *Biochem. Genet.* **16**, 883.
10. Tsai, C. Y., Huber, D. M. and Warren, H. L. (1980) *Plant Physiol.* **66**, 330.
11. Tsai, C. Y. (1983) *Plant Breeding Rev.* **1**, 103.
12. Glover, D. V. (1976) in *Improving the Nutrient Quality of Cereal II*, p. 69. Agency for International Development, Washington, DC.
13. Lee, L. (1982) Ph.D. Thesis, Purdue University, W. Lafayette, IN.
14. Tsai, C. Y. (1979) *Maydica* **24**, 129.
15. Lee, L. and Tsai, C. Y. (1984) *Plant Physiol.* **76**, 79.
16. Lee, L. and Tsai, C. Y. (1984) *Biochem. Genet.* **22**, 729.
17. Sodek, L. and Wilson, C. M. (1970) *Biochim. Biophys. Acta* **140**, 29.
18. Tsai, C. Y., Salamini, F. and Nelson, O. E. (1970) *Plant Physiol.* **46**, 299.
19. Roe, J. D. (1934) *J. Biol. Chem.* **107**, 15.
20. Nelson, N. (1964) *J. Biol. Chem.* **153**, 375.
21. Mertz, E. T., Misra, P. S. and Jambanathan, R. (1974) *Cereal Chem.* **51**, 304.
22. Salisbury, F. B. and Ross, C. W. (1978) *Plant Physiology*, p. 18. Wadsworth, Belmont.