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EVIDENCE FOR THE GENETIC CONTROL OF LYSINE CATABOLISM IN MAIZE ENDOSPERM

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Key Word Index—*Zea mays*; Gramineae; maize; lysine content; genetic regulation; maize endosperm.

Abstract—A split pollination was used to produce normal (*Su su su O2 o2 o2*) and high lysine double mutant *sugary opaque-2* (*su su su o2 o2 o2*) endosperms on the same ear of *sugary opaque-2* maize plants. Amino acids were determined in the vascular sap of the ear peduncle. Lysine content in the sap was compared with lysine stored in both normal and *sugary opaque-2* endosperm during kernel filling. Lysine content in the ear peduncle sap could account for all lysine found in both endosperms. Preformed lysine is highly catabolized in the normal endosperm, but not in the high lysine *sugary opaque-2* endosperm. The rate of lysine breakdown appears to be an important mechanism by which the high lysine mutant controls lysine level in maize endosperm.

INTRODUCTION

Since the discovery of the high lysine content in the endosperm of the *opaque-2* corn [1], considerable attention has been given to the effect of this gene on the synthesis of storage proteins. It has been shown that the 'major' effect of the *opaque-2* gene is a reduction in the amount of zein in the endosperm [1–4]. Since zein is essentially devoid of lysine, a reduction in zein brings about an increase in albumins, globulins and glutelins causing an increase in lysine in the endosperm. A high lysine mutation has a striking effect on lysine content in the endosperm as demonstrated both in maize [5] and in barley [6]. Incorporation of lysine-[^{14}C] in normal and high lysine *opaque-2* endosperms showed that in normal endosperm 14% of the injected ^{14}C ws converted to glutamic acid and 8% in proline, whereas in the high lysine *opaque-2* endosperm only 3% of ^{14}C was converted to glutamic acid and none to proline. Lysine in the endosperm may be derived from preformed lysine, as reported by Lawrence and Grant [7] in wheat. The present work is an attempt to elucidate the genetic control of lysine accumulation in maize endosperm.

RESULTS AND DISCUSSION

Figure 1 shows a comparison of nitrogen content in the double mutant and in the normal type as well as dry matter accumulation during endosperm filling. Nitrogen accumulation (Fig. 1A) is highly correlated with dry matter stored in the endosperm (Fig. 1B). Thus, the large discrepancies between the two genotypes for nitrogen accumulation may be attributed to the greater reduction in dry wt of the *sugary opaque-2* endosperm.

The peduncle sap analysis showed a concentration of 120 mg/ml of sucrose, 36 mg/ml of reducing sugars and 178 μg/ml of nitrate. This finding suggests that the peduncle sap extracted by our method contains both xylem and phloem sap. The presence of reducing sugars, however indicates either contamination of cell sap from the sectioned penduncle area or sucrose inversion during extraction.

Nitrogen and lysine content in the sap and in both double mutant and normal endosperms are shown in Table 1. Lysine in the vascular exudate was considered to be free lysine since no protein was found in the vascular sap [8]. The nitrogen content in vascular sap and endosperm decreased with kernel development. Lysine content in the sap, however, was maintained at constant levels with 4.6, 4.8, 4, 5 and 4.4 μmol% of total amino acid at 7, 14, 21, 28 and 35 days after pollination (DAP), respectively; our data seems to support the hypothesis of Lawrence and Grant [7]. Although some lysine synthesis may occur in the endosperm [9], lysine may be largely synthesized in other plant tissues and then transported to the kernels. The normal endosperm may receive a higher amount of preformed lysine than *sugary opaque-2* kernels since normal endosperm showed higher accumulation of nitrogen (Fig. 1A). However, lysine accumulation patterns in the two genotypes appear to be distinct (Table 1). Lysine content differs in the two endosperm genotypes commencing at 14 DAP. While normal endosperms show reduction of lysine concentration during maturation, the high lysine double mutant tends to maintain a constant level of lysine in the endosperm.

Based on the lysine content of the peduncle sap and calculating its flux into the endosperm from the rates of nitrogen accumulation, the amount of preformed lysine entering the endosperm may be estimated. This may be compared with the observed level of lysine actually incorporated into the endosperm at different stages of development. The observed and estimated lysine content in the double mutant and normal endosperms are shown in Fig. 2. A curvilinear regression analysis was used to construct the curves. The observed and estimated lysine per endosperm are quite similar for the double mutant

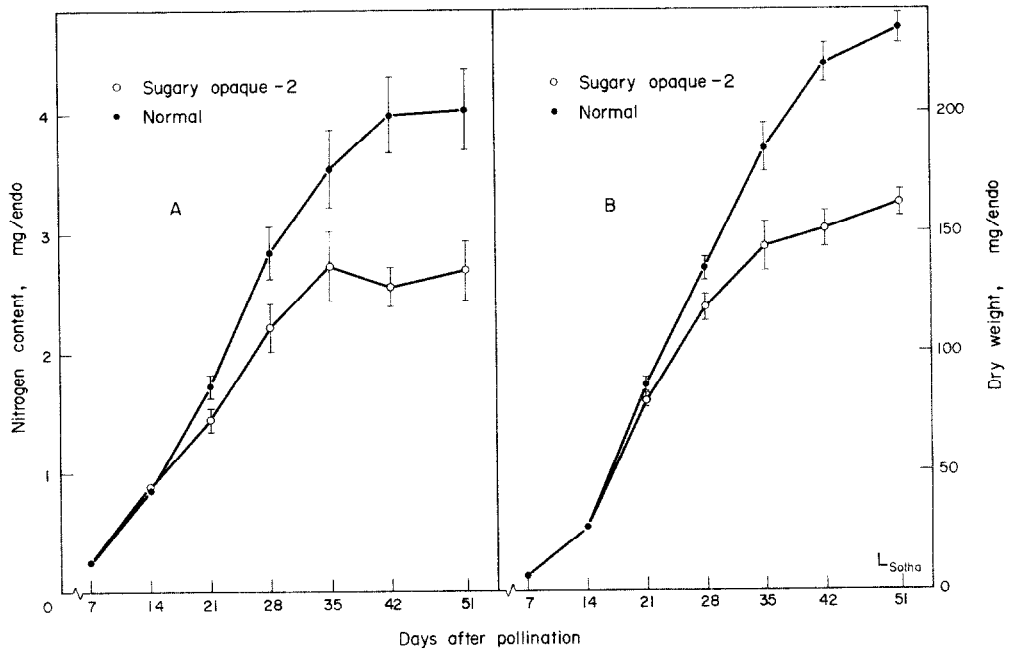

Fig. 1. Niteogen content (A) and dry weight (B) in normal and *sugary opaque-2* endosperm during kernel development. Bars indicate 95% confidence interval.

Table 1. Nitrogen and lysine content in vascular sap of ear peduncle, in *sugary opaque-2* mutant (*su o2*) and normal endosperms during kernel development

Material	Genotype	Component	Days after pollination						
			7	14	21	28	35	42	51
Vascular sap	*su o2*	Total nitrogen (mg/ml)	1.7	1.5	1.6	1.4	1.4	1.3	*
		Lysine (μmol%)	4.6	4.8	4.0	5.0	4.4	2.7	*
Endosperm	*su o2*	Total nitrogen (% of dry matter)	4.0	3.5	1.8	1.9	1.9	1.7	1.7
	Normal		3.9	3.3	2.0	2.1	1.9	1.8	1.7
	su o2	Lysine (μmol%)	3.4	3.5	4.0	3.7	3.4	3.5	4.2
	Normal		3.4	3.0	2.4	1.8	1.9	1.5	1.4

* Peduncles were dry.

endosperm (Fig. 2A). However for normal endosperm the estimated and observed values are very different. Estimated values can be as high as 2.5 times the observed lysine content after the third week of endosperm filling (Fig. 2B). Thus, the excess lysine entering the normal endosperm must be catabolized from a very early stage in the normal endosperm: on the other hand, in the high lysine endosperm, breakdown of lysine would not be necessary except perhaps at later stages of development.

Our data are in agreement with the results observed in maize [5] and barley [6] indicating that catabolism of lysine is higher in the normal endosperm than in the high lysine endosperm mutant. We conclude that the rate of lysine conversion may be an important mechanism for the control of lysine levels in maize endosperm.

EXPERIMENTAL

A synthetic double mutant *sugary opaque-2* (*su o2*) maize variety was used as the female parent in this study. The *su o2* plants were grown in adjacent 100 cm rows at a density of 50 000 plants/ha. Plants were grown in the experimental field at the Universidade Estadual de Campinas, São Paulo, Brazil. The plots were fertilized with N, P_2O_5 and K_2O at 60, 80 and 30 kg/ha, respectively. Through a split pollination technique the double mutant plants were selfed and outcrossed with a normal endosperm variety which was used in the synthesis of the double mutant variety [10]. Normal (*Su su Su2 o2 o2*) and *sugary opaque-2* (*su su su o2 o2 o2*) endosperms were produced on the same ear in a proportion of 1:1 in *ca* 105 adjacent plants.

Fifty kernels from the middle portion of the ear were sampled

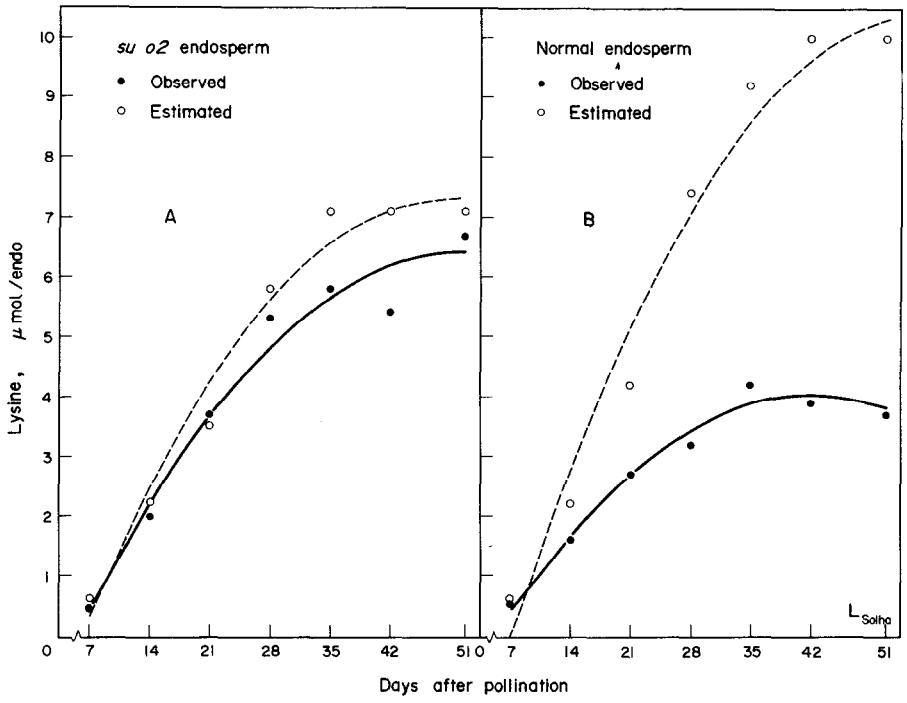

Fig. 2. Observed and estimated lysine content in *sugary opaque-2* (A) and normal (B) endosperm during kernel development.

from each genotype in 7 different plants (replications), taken at 7, 14, 21, 28, 35, 42, 51 DAP. Physiological maturity was attained when kernels reached 42–51 DAP with seed black layer formation. The ear penduncle for sap analysis was taken from each split pollinated plant whose ear was used for endosperm analysis. Ten peduncles *ca* 5 cm long were cut from the base of the ear and vascular sap was extracted for each stage of endosperm development. The vascular sap was extracted from the ear peduncle as follows: the peduncle was attached to the top of a test-tube and 0.5 atm vacuum was applied to extract the vascular sap. At the same time, 5 ml $CHCl_3$ was passed through the peduncle to facilitate extraction. This process was repeated for each peduncle and the final extract was separated from $CHCl_3$ by centrifugation at 1000 *g* to facilitate phase separation. To test whether the exudate represents either xylem or phloem sap or both, the method established in refs. [11, 12] was used. Thus in the exudate collected from the ear penduncle, sugar content (phloem sap indicator) and nitrate (xylem sap indicator) were determined. Reducing sugars and sucrose were determined by difference in total sugars before and after hydrolysis with N HCl by the 3,5-dinitrosalicylic acid method [13]. Nitrate was determined by the phenoldisulfonic acid method [14].

Forty kernels of each genotype at each stage of development were degermed and stored at −20°. The endosperms were then lyophilized and ground to powder. Endosperm and vascular sap N determinations were carried out in a Technicon Auto Analyser [15]. For endosperm lysine analysis, 7 replications were pooled. The pooled endosperm samples and vascular sap were analysed for lysine with an amino acid analyser ref. [16].

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