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PROTEIN AND FREE AMINO ACIDS IN A HIGH LYSINE MAIZE DOUBLE MUTANT

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Key Word Index—*Zea mays*; Gramineae; protein synthesis; free amino acids; high lysine endosperm mutants.

Abstract—A comparative study of free amino acids and protein fractions of normal with a double mutant (su_1o_2) was made, during endosperm development in segregating ears of a maize synthetic. Zein content showed striking differences in the two genotypes, being 7.7 and 6 times greater in the normal endosperm at 24 and 47 days after pollination respectively. This observed decrease in zein synthesis, coded by sugary-1/opaque-2 genes, causes an accumulation of alanine, glutamic and aspartic acids, glutamine and asparagine in the high lysine endosperm mutant.

INTRODUCTION

Although total free amino acids in corn and other cereals have been reported [1] there has been relatively little emphasis upon the association of free amino acid composition with protein synthesis in kernels [2–4].

Research in this area has involved either normal endosperm, analysed in several stages of endosperm development [3], or restricted to a comparison of normal and opaque-2 endosperm in mature kernels [4].

This study is an attempt to correlate free amino acid composition and protein synthesis in corn endosperm through a comparitive study of normal with a high lysine double mutant, sugary-1/opaque-2 during kernel development.

RESULTS AND DISCUSSION

The distribution of nitrogen among the protein fractions of endosperm is reported in Table 1. Total nitrogen differed in the two endosperms only at 47 days after pollination (DAP), being 58.4 % higher in the normal kernels.

Free amino acids, albumin and globulin increased in both endosperms from 12 to 24 DAP, and decreased from 24 to 47 DAP. The content was always higher in the double mutant endosperm after 24 DAP. The zein content increased only after 24 DAP, being the major component of the protein fraction at 47 DAP in normal endosperm. The double mutant depressed drastically the rate of zein synthesis during kernel development, but had no effect on glutelin synthesis (GII and GIII). Glutelin I fraction, however, was higher in normal endosperm at 24 and 47 DAP. The difference between su_1o_2 and normal endosperm for total nitrogen at 47 DAP is attributed mainly to the zein fraction. Free amino acid composition in μmol/endosperm in both normal and double mutant endosperm (Table 2) was determined. In addition, free amino acid composition was compared with the protein fractions during kernel development.

In all three stages of development 12 out of 16 amino acids showed higher concentrations per endosperm in the double mutant at 24 and 47 DAP. These differences might be attributed to the fact that sugary-1/opaque-2 endosperm is *ca* two times higher in free amino acids

Table 1. Nitrogen distribution in protein fractions of both sugary-1/opaque-2, (su_1o_2) and normal corn endosperms, during kernel development (μmol/endosperm)

DAP	12		24		47	
Types	su_1o_2	normal	su_1o_2	normal	su_1o_2	normal
Protein fractions						
Faa	19.0	19.4	49.8	24.3	28.5	16.6
I Alb + Glob	10.1	10.9	56.7	36.9	27.2	21.9
II Zein	0.2	0·1	6·5	50·5	28.5	168.0
III G I	0.1	0.1	4.8	11.1	19.7	31.3
IV G II	0.2	0.4	7.8	8.0	20.4	18.8
V G III	2.2	2.4	13.8	14.5	48.9	44.1
Total N	33.0	34.5	141.7	147.7	182.6	312.8

DAP = days after pollination; Faa = free amino acids; Alb = albumin; Glob = globulin; GI = glutelin I; G II = glutelin II; G III = glutelin III.

Table 2. Free amino acid composition (μmol/endosperm) of surgary-1/opaque-2 (su_1o_2) and normal endosperms during kernel development

DAP	12		24		47	
Types	su_1o_2	normal	su_1o_2	normal	su_1o_2	normal
Lys	0.19	0.26	0.99	0.75	0.79	0.60
Hys	0 16	0.17	—	—	—	—
Arg	0.13	0.22	0.20	0.34	0.30	0.21
Asp	0.97	0.68	2.23	1.73	5.11	2.69
Thr	0.22	0.21	0.76	0.56	0.44	0.33
Ser	0.45	0 43	1.59	0.89	1.08	0.58
Glu	0.68	0.61	7.17	2.00	4.08	2.00
Pro	1.77	1.70	0.80	0.47	1.22	0.79
Gly	0.17	0.26	1.03	0.64	0.28	0.34
Ala	5.30	4.76	12 40	5.15	4 95	3.01
Val	0.32	0.36	1.47	0 47	0.44	0.20
Met	0.06	0.07	0.36	0.34	—	—
Cys	—	—	—	—	—	—
Ilcu	0.07	0.10	0.44	0.19	0 26	0.16
Leu	0.10	0.12	0.44	0.36	0.26	0.17
Tyr	0.10	0.11	0.60	0.19	0.54	0.18
Phe	0.12	0.12	0.16	0.19	0.16	0 12
AsN	0.82	1.23	2.43	0.52	2.11	1 14
GlN	2.80	2.46	6.33	3.41	1.31	0.85
Trp	—	0 12	—	0.19	—	—

DAP = days after pollination

than the normal counterpart. Significant differences, however, were found for alanine, glutamic acid, aspartic acid, glutamine and asparagine with each showing higher concentration in the high lysine double mutant. As these amino acids are important components of zein [4–7], our data suggest that sugary-1/opaque-2 genes depress zein synthesis with consequent accumulation of alanine, glutamic and aspartic acid, glutamine and asparagine. A reduction in zein synthesis in the double mutant, should yield an accumulation of leucine and proline in the endosperm. However, an increased yield was not attained, possibly due to a low rate of syntheiss of proline and leucine observed in the high lysine double mutant endosperm (unpublished data).

We can also conclude that lysine content in sugary-1/opaque-2 endosperm is mainly determined by albumins and globulins at milk stage (40.1% of N at 24 DAP) and albumins and globulins plus glutelin III near kernel physiological maturity (41.7% of N at 47 DAP).

EXPERIMENTAL

A synthetic double mutant sugary-1/opaque-2 corn variety designated Nutrimaiz developed by Silva *et al.* [8] was used as the female parent in this study. This germplasm was self pollinated and crossed with a normal endosperm variety which was used in the synthesis of Nutrimaiz [8].

Normal and double mutant endosperms were produced in segregating ears, using the double pollination technique as previously described [9]. Kernels for analysis were collected at 12, 24 and 47 days after pollination from 10 segregating ears and stored frozen. Kernels were lyophilized, degermed and the endosperms were ground to powder using a Spexmill.

Proteins were extracted from endosperm using the technique described in ref [10]. The amount of total N in each of the 5 fractions was determined by the micro-Kjeldahl method. Free amino acids were separated from fraction I by precipitation with 5% TCA. Amino acid composition was determined with an automatic amino acid analyser using a ligand system.

Amide content of the free amino acid fractions was determined by difference in aspartic and glutamic acid contents before and after hydrolysis with 1N HCl for 3 hr at 105° [4].

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REFERENCES

1. Misra, P. S., Mertz, E T. and Glover, D. V. (1975) *Cereal Chem.* **52**, 844.
2. Brandt, A. B. (1975) *FEBS Lett.* **52** (2), 288
3. Moureaux, T. and Landry, J. (1972) *Physiol. Veg.* **10**, 1.
4. Sodek, L and Wilson, M. C. (1971) *J. Agric. Food Chem.* **19**, 1144.
5. Christianson, D. D., Nielson, H. C., Khoo, U., Wolf, M. J. and Wall, J. S. (1969) *Cereal Chem.* **46**, 372.
6. Mossé, J., Baudet, J., Landry, J. and Moureaux, T. (1966) *Ann. Physiol. Veg.* **8**, 331.
7 Paulis, J. W., James, C. and Wall, J. S. (1969) *J Agric. Food Chem* **17**, 1301.
8. Silva, W. J. da, Teixeira, J. P. F., Arruda, P and Lovato, M. B (1977) *Crop Sci* (submitted).
9. Silva, W. J. da, Lovato, M. B. and Arruda, P. (1977) *Crop Sci* (submitted).
10. Landry, I. and Moureaux, T. (1970) *Bull. Soc. Chim Biol.* **52**, 1021