Activity of nitrogen metabolism enzymes in the process of kernel development in different maize genotypes

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The activity of GDH, GOT, GPT, lysine-ketoglutarate reductase and GS in the developing endosperm of normal maize, opaque-2, sugary-2 and opaque-2 sugary-2 mutants was investigated. The results indicate that the o_2 gene increases GDH, GOT and GPT activities and decreases lysine catabolism in the developing endosperm. The presence of different mutants in the same genetic background does not affect GS activity.

Glutamate dehydrogenase *Aspartate aminotransferase* *Alanine aminotransferase*
Glutamine synthetase *Lysine-ketoglutarate reductase* *Maize mutant*

1. INTRODUCTION

Since the discovery of high lysine mutants, which have a high nutritional value, there has been increasing interest in studying biochemical changes leading to increased lysine content and an altered proportion of amino acids in the endosperm.

High lysine maize endosperm has altered protein composition and increased lysine content [1].

Besides prolamine synthesis in the endosperm being decreased, major differences exist in the metabolism in normal and high lysine varieties; it has been shown that high lysine varieties of barley as well as corn have an altered amino acid metabolism [2–5]. The mechanism leading to such an altered proportion of amino acids is not known.

Glutamine, and to a lesser extent asparagine, are thought to be the major transport forms of nitrogen in cereal seeds [6] and developing seeds are able to synthesize many of the amino acids required for the formation of their storage proteins [7–9].

Abbreviations: GS, glutamine synthetase; GDH, glutamate dehydrogenase; GOT, aspartate aminotransferase; GPT, alanine aminotransferase; o_2, opaque-2; su_2, sugary-2

Since in plants all amino acids originate from either glutamine or asparagine and since glutamate, aspartate and alanine play the key role in amino acid metabolism, it was of interest to determine GS, GDH, GOT and GPT activity in the developing normal and mutant maize endosperm.

Increased lysine content in the endosperm of mutants is due neither to transport of higher lysine amounts into the developing kernel [5], nor to changes in enzyme regulation in lysine synthesis [10].

High lysine content in mutant maize endosperm is due to a lower rate of lysine catabolism to proline and glutamate [3,5,9]. According to authors in [11], lysine-ketoglutarate reductase is involved in lysine catabolism in maize endosperm. This paper reports the activities of this enzyme during the development of normal and mutant maize endosperm.

2. MATERIALS AND METHODS

2.1. *Plant material*

Inbred seeds of Oh 43 normal, Oh 43 opaque-2, Oh 43 sugary-2 and Oh 43 opaque-2 sugary-2 were planted in a field at Zemun Polje in 1982. All plants were self-pollinated. Ears were harvested in

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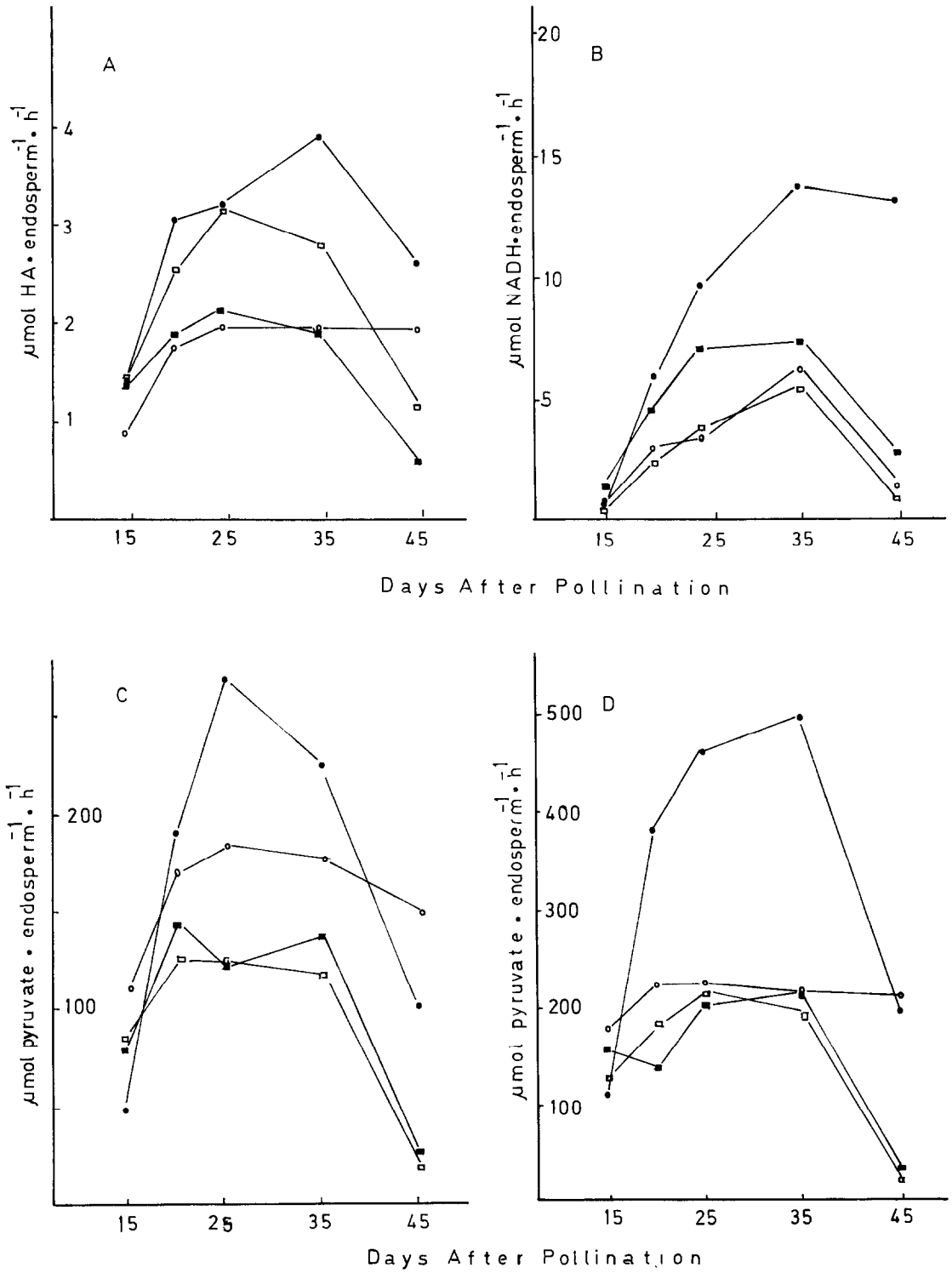

Fig.1. Changes in level of enzymes of nitrogen metabolism. GS (A), GDH (B), GOT (C), GPT (D) in developing endosperm of normal (○), opaque-2 (●), sugary-2 (□) and opaque-2 sugary-2 (■) maize.

triplicate at regular intervals from 15 to 45 days after pollination. Immature ears were immediately frozen and stored at −20°C until use.

2.2. *Enzyme determinations*

For the determination of enzyme activity, crude enzyme preparations from endosperm tissue were used.

GS activity was determined as in [12], GDH activity according to [13], aminotransferases (GOT and GPT) as in [14] and lysine-ketoglutarate reductase according to [15].

The final activity of each enzyme was calculated on the basis of the amount of product produced or substrate utilized/endosperm per h.

3. RESULTS AND DISCUSSION

All the enzymes investigated were present in the immature endosperm in each variety (fig.1A–D, 2). There is a general increase in the activity in the period of intensive storage-protein synthesis and accumulation of total nitrogen.

GS activity was higher in the opaque-2 mutant than in the normal endosperm throughout the experimental period, but that was not the case with the opaque-2 sugary-2 mutant and its sugar-2 counterpart (fig.1A). These results, and our previous results for opaque-2 maize hybrids [16] and synthetics [17] and those of authors in [18] suggest that the presence of different mutants in the same genetic background does not show any regular changes in GS activity.

Low GS activity (up to 4 μmol glutamylhydroxamate · endosperm^{-1} · h^{-1}) confirms that glutamine synthesis is not essential for endosperm development, because glutamine reaches the kernel by transport from the vegetative parts of the plant.

Our results show that the level and pattern of development of GDH, GOT and GPT differ significantly in opaque-2 mutants from the normal variety, but only slightly in the opaque-2 sugary-2 mutant compared with the sugary-2 variety (fig.1B–D).

GDH activity in the endosperm of the opaque-2 mutant is much higher than in the normal during the period of intensive synthesis of storage proteins (fig.1B).

It is known that NH_4^+ levels in the normal maize endosperm increase just prior to the onset of zein biosynthesis and then decline. In opaque-2, NH_4^+ levels are higher than normal initially and remain high throughout the experiment [18]. Higher GDH activities in opaque-2 endosperm suggest increased glutamic acid synthesis from NH_4^+ and 2-ketoglutarate. This synthesis continues after the 45th day after pollination. Authors in [18] demonstrated the same trend for glutamic acid accumulation in normal and opaque-2 endosperm, but could not detect any changes in GDH activity.

Both transaminases investigated (GOT and GPT) are more active in opaque-2 endosperm from 20 to 35 days after pollination. The transamination processes in opaque-2 endosperm abruptly intensify after 15 days, reach their maximum on the 25th day (GOT) and 35th day (GPT) after pollination, respectively, and abruptly decline 45 days after pollination. In normal endosperm the same procsses occur at a more uniform rate during the period studied (fig.1C,D).

In maize endosperm the activities of GPT exceed those of GOT. High activities of GOT and GPT in the endosperm during development correlate with high relative amounts of aspartic acid and alanine in the endosperm.

Increased activities of lysine-ketoglutarate reductase coincide with the intensification of the metabolism process in the kernel for the synthesis of kernel storage proteins (fig.2). Normal maize endosperm synthesizes high amounts of zein with minimum lysine content. Considering that this protein makes up about 50% of total protein in the maize kernel, lysine is subject to metabolism more than in opaque-2 mutant endosperm, in which zein synthesis is retarded and the amount of other protein fractions containing higher amounts of lysine is proportionately increased.

The results obtained (fig.2) show that the presence of the o_2 gene in maize endosperm leads to a decline in activity of lysine-ketoglutarate reductase, i.e., this enzyme controls the lysine level in maize endosperm.

The presence of the su_2 gene in the Oh 43 genetic background causes decreased starch and zein contents in the kernel [19] and, therefore, decreases kernel weight. The kernel undergoes the development stages faster, so protein synthesis terminates earlier. The processes investigated are, therefore, less intensive in the double mutant than in the opaque-2 mutant.

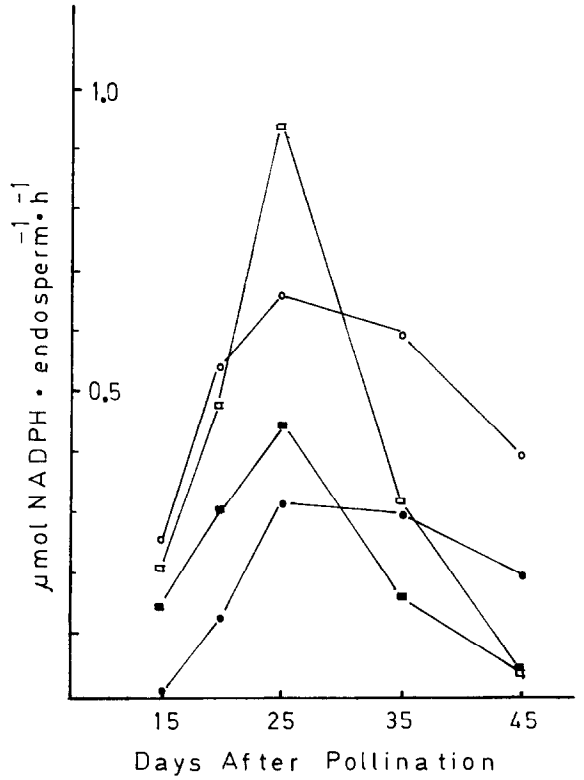

Fig.2. Lysine-ketoglutarate reductase activity in normal (○), opaque-2 (●), sugary-2 (□) and opaque-2 sugary-2 (■) maize endosperm.

The present results indicate that the o_2 gene affects some changes in the biosynthesis of the soluble precursors of protein synthesis in the developing maize endosperm.

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REFERENCES

[1] Nelson, O.E. (1969) Adv. Agron. 21, 171–194.
[2] Sodek, L. (1970) Arch. Biochem. Biophys. 140, 29–38.
[3] Brandt, A.B. (1975) FEBS Lett. 52, 288–291.
[4] Dalby, A. and Tsai, C.Y. (1975) Crop Sci. 15, 513–515.
[5] Da Silva, W.J. and Arruda, P. (1979) Phytochemistry 18, 1803–1805.
[6] Arruda, P. and Da Silva, W.J. (1979) Phytochemistry 18, 409–410.
[7] Kolderup, F. (1979) in: FAO/IAEA Symposium on Seed Protein Improvement in Cereals and Legumes, vol.1, International Atomic Energy Agency, Vienna, pp.187–202.
[8] Sodek, L. (1976) Phytochemistry 15, 1903–1906.
[9] Sodek, L. and Wilson, C.M. (1970) Arch. Biochem. Biophys. 140, 29–38.
[10] Dierks-Ventling, C. (1983) Planta 157, 233–238.
[11] Arruda, P., Sodek, L. and Da Silva, W.J. (1982) Plant Physiol. 69, 988–989.
[12] Elliot, W.H. (1953) J. Biol. Chem. 201, 661–672.
[13] Schmidt, E. (1970) in: Methoden der Enzymatischen Analyse (Bermeyer, H.U. ed) pp.607–613, Academic Verlag, Berlin.
[14] Tonhazy, N.E., White, N.G. and Umbert, W.W. (1970) in: Methoden der Enzymatischen Analyse (Bergmeyer, H.U. ed) pp.695–698; 727–731, Academic Verlag, Berlin.
[15] Hutzler, J. and Dancis, J. (1968) Biochim. Biophys. Acta 158, 62–69.
[16] Hadži-Tašković Šukalović, V. (1983) Arhiv za Polj. Nauk. 43, 279–299.
[17] Hadži-Tašković Šukalović, V. and Petrović, R. (1982) Genetika 14, 227–243.
[18] Misra, S. and Oaks, A. (1981) Can. J. Bot. 59, 2735–2743.
[19] Dalby, A. and Tsai, C.Y. (1975) Crop Sci. 15, 513–515.