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METABOLISM OF ACETATE-^{14}C IN NORMAL AND OPAQUE-2 *ZEA MAYS* ENDOSPERM DURING DEVELOPMENT*

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Key Word Index—*Zea mays*; Gramineae; maize, opaque-2; normal; acetate metabolism; grain development.

Abstract—Acetate-[2-^{14}C]metabolism by developing normal and opaque-2 maize endosperms showed considerable differences in incorporation of label into organic acids, protein and free amino acids. Protein synthesizing efficiency was higher in opaque-2 endosperm 15 days after pollination but in normal at later stages of development. The differences in incorporation of label were more pronounced at early stages of endosperm development. Differences between the two endosperms occurred in the labelling of aspartate and glutamate in the free amino acids at 15 days post-pollination and in protein amino acids at 25 days post-pollination. Label and specific activity in protein lysine was higher in opaque-2 than in the normal.

INTRODUCTION

Depressed zein synthesis in opaque-2 maize is mainly responsible for higher lysine and tryptophan and an improved leucine–isoleucine ratio [1–5] since it is deficient in lysine and rich in leucine. The opaque-2 gene appears to act not by modifying any major storage protein [6] but by exerting a regulatory control on the synthesis of *m*RNA required for zein formation at early stages of development [7]. Sodek and Wilson [8] showed differences in amino acid metabolism of developing normal and opaque-2 maize endosperms. Considerable differences in RNase activities [9–11], *in vitro* incorporation of leucine and lysine [7,12], soluble protein pattern [13] and RNA polymerase [14,15] between normal and opaque-2 endosperm during development have also been shown. In the present study the pattern of acetate-[2-^{14}C]metabolism in developing endosperm of normal and opaque-2 maize has been studied in order to determine the biochemical differences between them.

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RESULTS

Incorporation of label into different endosperm fractions

The distribution of label from acetate-[2-^{14}C] into soluble sugars, organic acid, free and protein amino acid, and lipid fractions of developing normal and opaque-2 endosperm is shown in Table 1. At the 15-day stage the total label incorporated per endosperm in opaque-2 was more than double that of the normal but at other stages differences were small. Most of the label was incorporated into free amino acids and organic acids. The endosperms differed considerably in the proportion of the label incorporated into free amino acids and protein amino acids. At the 15-day stage, per cent label incorporated into protein amino acids of opaque-2 was about 3 times greater than the normal but this trend was reversed at later stages. A substantially higher label

Table 1. Distribution of label (%) incorporated in different fractions of developing normal and opaque-2 maize endosperm

Fraction	Days after pollination					
	15		20		25	
	N*	O†	N	O	N	O
Ethanol soluble–ether soluble	3.56	2.63	5.27	3.69	0.22	0.41
Ethanol insoluble–ether soluble	0.77	1.07	1.17	0.62	1.01	0.77
Soluble sugars	2.50	1.70	1.68	1.02	1.34	1.01
Organic acids	23.29	50.20	24.54	22.53	30.61	24.69
Free amino acids	64.56	28.16	53.42	62.54	50.76	64.53
Protein amino acids	5.32	16.25	13.93	9.61	16.06	8.59
Total counts (cpm endosperm)	6.48×10^5	14.75×10^5	11.37×10^5	10.66×10^5	12.44×10^5	13.81×10^5
Ratio of radioactivity in free amino acids to protein amino acids	1:0.08	1:0.58	1:0.26	1:0.15	1:0.32	1:0.13

* N = Normal. † O = Opaque-2.

Table 2. Distribution of label in different organic acids as % of total label in this fraction at various stages of development of normal and opaque-2 maize endosperm

		Days after pollination					
		15		20		25	
$R_f \times 100$	Organic acid	N	O	N	O	N	O
5	Citric	7.35	8.25	8.47	5.08	3.45	10.69
12–12.5	Oxaloacetic	4.83	9.80	7.72	6.07	2.81	6.69
26–27	Malic	54.78	64.71	57.39	37.86	64.58	46.05
30–31	Succinic	21.00	11.73	18.92	34.34	21.78	20.92
53	Unidentified	3.36	2.26	2.83	6.66	A	A
60	Unidentified	A*	A	A	A	7.38	15.64
67	Unidentified	8.68	3.25	4.67	9.99	A	A

* A = Organic acid of corresponding R_f absent.

appeared in free amino acids. Both endosperms incorporated more than 50% of the label into free amino acids at all the stages except at the 15-day stage where it was only *ca* 28% for opaque-2. At 20 and 25 day stages opaque-2 incorporated more label into the free amino acids than the normal.

If protein synthesizing efficiency is expressed as the proportion of label in protein amino acids to free amino acids, then it is 0.08 for normal compared with 0.58 for opaque-2 at the 15-day stage. In other words at this stage opaque-2 was *ca* 7 times more efficient than the normal in its protein synthesizing ability. However, at the 20 and 25 day stages normal endosperms had 1.7 and 2.5 times respectively more protein synthesizing efficiency than opaque-2.

At the 15-day stage, opaque-2 endosperm incorporated more label into organic acids than the normal but at later stages the differences were small. The proportion of label incorporated into EtOH-soluble Et_2O-soluble, EtOH-insoluble Et_2O-soluble and soluble sugars did not show much difference between normal and opaque-2 during development. The total label incorporated into these three fractions was found to be less than 9%.

Distribution of label in different organic acids

Results presented in Table 2 show the distribution of label in different organic acids as a % of the total label in this fraction at different stages of endosperm development. Most of the label appeared in malic acid followed by succinic acid at all the stages. The label incorporated into malic acid during endosperm development increased from 54.8 to 64.6% in normal whereas it decreased considerably in opaque-2. Normal endosperm at the 15-day stage had a lower % label in malic acid than that of opaque-2, but at later stages this was reversed.

The proportion of label incorporated into succinic acid of normal endosperm was more or less constant whilst it differed considerably in opaque-2 during development. At the 15-day stage normal endosperms had almost two times more label in succinic acid than that of opaque-2. However, the trend was reversed at the 20-day stage and at the 25-day stage the difference was negligible. The proportion of label incorporated into oxaloacetate and citrate was higher in opaque-2 than that of the normal at the 25-day stage. TLC revealed that the organic acids of R_f 0.67 and 0.60 had the maximum concentration and oxaloacetate had the minimum concentration in the organic acid fraction. Malate ranked second and citrate third with respect to their relative proportions. The relative proportion of the different organic acids was similar in the normal and opaque-2 endosperms during development.

Distribution of label in free amino acids

Results presented in Table 3 show considerable differences in the proportion of label incorporated into aspartate, glutamate and neutral amino acids at the 15-day stage in normal and opaque-2 endosperms whilst at other stages they were less. Normal endosperm had 53.4% of the label in glutamate as against only 3.4% in opaque-2 at the 15 day stage, while opaque-2 had 2.3 times as much label in the neutral amino acids. The labelling of aspartate was *ca* 4 times greater in normal than that in opaque-2 at the 15-day stage but at other

Table 3. Distribution of label in free amino acids as % of total in this fraction in normal and opaque-2 maize endosperm at different stages of grain development

	Days after pollination					
	15		20		25	
Amino acid	N	O	N	O	N	O
Aspartic acid	5.65	1.30	13.07	15.83	16.82	18.67
Glutamic acid	53.40	3.44	36.52	41.88	31.26	28.05
Neutral	40.68	95.18	50.32	42.16	51.89	53.26
Histidine	0.03	0.04	0.02	0.05	0.01	Traces
Arginine	0.11	0.03	0.03	0.04	Traces	0.01
Lysine	0.14	0.03	0.05	0.03	0.03	0.01

Table 4. Distribution of label in different protein amino acids as % of total label in this fraction in normal and opaque-2 maize endosperm at different stages of grain development

	Days after pollination					
	15		20		25	
Amino acid	N	O	N	O	N	O
Aspartic acid	20.84	18.57	18.76	19.62	17.66	20.06
Glutamic acid	50.99	53.26	43.60	50.82	52.92	43.82
Neutral	21.86	22.35	31.75	25.08	26.18	31.02
Histidine	0.60	0.30	0.38	1.29	0.35	0.58
Arginine	4.91	4.18	5.03	1.95	2.77	3.72
Lysine	0.79	1.34	0.47	1.24	0.13	0.80

stages the differences were less. Histidine, arginine and lysine accounted for less than 1% of the total label. The ratio of label in glutamate and aspartate was 9.5 in normal compared with 2.7 in opaque-2 at the 15-day stage. However, at the 20 and 25-day stages the ratios were more or less equal in normal and opaque-2.

Distribution of label in different protein amino acids

Glutamate had the highest proportion of label in both normal and opaque-2 endosperms during development (Table 4). The labelling of glutamate decreased in opaque-2 during development but in the normal it decreased at the 20-day stage and then increased at the 25-day stage. The ratio of labelling in glutamate to aspartate altered only slightly during development. The labelling of aspartate and the neutral amino acids was similar in both endosperms but that of lysine was higher in opaque-2 than that of the normal at all stages. During development it decreased in both endosperms, the largest decrease occurring in the normal.

Specific activity of free and protein amino acids

The amount and sp. act. of aspartate, glutamate and lysine in free and protein amino acids fractions at 15 and 25-day stages are given in Table 5. In the free amino acid fraction sp. act. of all three amino acids was much higher in normal than that of opaque-2 at the 15-day stage. The lower sp. act. values in opaque-2 endosperm are probably due to the higher amounts of these amino acids at this stage. The very low sp. act. of aspartate and glutamate in opaque-2 cannot be solely accounted for by the higher levels of these amino acids as it has been seen earlier (Table 3) that at this stage most of the label appears in neutral amino acids. It is likely that either there is a very fast conversion of glutamate and aspartate to other amino acids or there is a greater synthesis of glutamine and asparagine which would be included in the neutral amino acid fraction. The latter seems more likely on the basis of the sp. act. values for protein amino acids since in this fraction asparagine and glutamine would be included together with aspartate and glutamate because of acid hydrolysis.

At 25 days post-pollination the sp. act. of aspartate was higher in opaque-2 endosperm and that of glutamate in normal. The concentration of glutamate, aspartate and lysine in opaque-2 was *ca* twice as much as that in normal.

On comparing the sp. act. of protein amino acids it is seen that the values for glutamate and aspartate were higher at the 15-day stage and lower at the 25-day stage in opaque-2 endosperms when compared with normal. The ratio of the amount of glutamate to aspartate was found to be the same for both endosperms at the 15-day stage while at the 25-day stage it was slightly higher in opaque-2. The higher levels of lysine together with a higher sp. act. indicate a higher rate of incorporation of lysine in opaque-2 endosperm proteins compared with normal.

DISCUSSION

The metabolism of acetate-[2-^{14}C] by developing normal and opaque-2 maize endosperm showed considerable differences in net protein synthesizing ability, incorporation of label into free amino acids, protein amino acids and organic acids. These together with diverse metabolic changes observed by other workers [1,4,7–9,11–14] suggest that the opaque-2 single recessive gene could have a regulatory role whereby it affects in turn a wide range of other genes. The metabolic differ-

Table 5. Specific activities of free and protein amino acids of developing normal and opaque-2 endosperms

Days after pollination	Amino acid	Specific activity (cpm × 10^2/μmol)				Amount (μg/endosperm)			
		Free amino acid		Protein amino acid		Free amino acid		Protein amino acid	
		N	O	N	O	N	O	N	O
15	Aspartate	356	20	6.51	9.65	60	189	435	599
	Glutamate	1447	65	12.59	22.12	153	221	609	827
	Lysine	16	6.6	0.41	1.04	34	50	290	440
25	Aspartate	653	743	4.65	4.28	139	245	754	660
	Glutamate	1004	759	8.93	4.84	178	399	1288	1397
	Lysine	2.8	1.3	0.17	0.31	38	92	460	550

ences observed were greater at early stages of endosperm development and this is consistent with the suggestion that the opaque-2 gene is active only during first 3 weeks after pollination [4,7]. The general pattern of distribution of label in organic acids, free amino acids and protein amino acids in normal and opaque-2 endosperm agrees well with the higher rate of protein deposition observed in opaque-2 endosperm during early development. The amount and distribution of label in lysine in the free and protein amino acids, indicate a higher rate of lysine biosynthesis and incorporation in protein in opaque-2 endosperm. During grain development more than 90% of the label appeared in organic acids and free and protein amino acids as against 50% during germination in maize [16] and barley [17]. The acetate carbon appeared very rapidly in free and protein amino acids in developing endosperms suggesting the presence of various enzymes of carbohydrate and protein metabolism in endosperm. The proportional distribution of label and sp. act. of free and protein amino acids indicate differences in amino acid metabolism in normal and opaque-2 endosperm.

EXPERIMENTAL

One of the high combining, well adapted inbred line Fla 3H 94-f-#-# available in the maize programme and its opaque version were used for the present study. The opaque version was obtained after 3 back crosses. Opaque and normal lines were grown at the I.A.R.I., New Delhi during the monsoon season of 1974. The self-pollinated ears were harvested at the specified dates after pollination. The harvested ears were immediately chilled, dissected and the endosperms collected.

Acetate-[2-^{14}C] feeding. Ten fresh endosperms at different stages of development were taken in a small sterilised beaker and 5 μl acetate-[2-^{14}C]sol (1 μCi, 23 mCi/mmol) per endosperm was added and incubated in the light for 3 hr at 25°. Usually all the radioactive soln was taken up in the first 30 min after which 10 μl H_2O per endosperm was added. Endosperms were then washed quickly first with a 0.1% soln of NaOAc and then with H_2O, blotted dry and stored in liquid N_2 immediately.

Extraction and fractionation. The methods used were similar to those used in refs [16,18]. Three endosperms in duplicates were extracted with boiling 80% EtOH by grinding in a mortar and pestle. The ground material was centrifuged at 20000 *g* for 20 min, the supernatant collected and the residue re-extracted × 3 with 10 ml 80% EtOH which extracted amino acids and sugars. The pellet was lyophilized and supernatants pooled, evaporated to dryness under vacuum at 40°. The soluble fraction thus obtained was further fractionated after dissolving in H_2O, into a $CHCl_3$ soluble fraction, organic acid, sugar and amino acid fractions by the methods of refs. [19,20]. Lipids were extracted from the pellet fraction and a portion was hydrolysed with 6 N HCl under vacuum in sealed tubes for 24 hr at 110°. The hydrolysate was filtered, evaporated to dryness under vacuum at 40°. The residue was dissolved in buffer and the pH adjusted to 6.2.

Amino acid analysis. The separation and estimation of basic, acidic and neutral amino acids in protein and free amino acid fractions was done by high voltage electrophoresis [21]. Lysine was also estimated by the colorimetric method of ref. [22].

Organic acids analysis. The acids were first converted to their NH_4 salts, separated by TLC [23] using cellulose powder and detected by spraying the plates with a 0.04% aq soln of bromo-cresol purple (pH 7). For measurement of activity, the individual spots were removed from the plate and counted.

Counting. The incorporation into each fraction was obtained by liquid scintillation counting in Bray's mixture and counts were corrected for quenching.

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