Phytochemistry, 1972, Vol. 11, pp. 937 to 942. Pergamon Press. Printed in England.

CHANGES IN THE NUCLEIC ACID AND PROTEIN FRACTIONS IN OPAQUE-2 MAIZE KERNELS DURING DEVELOPMENT

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(*Received* 14 *April* 1971)

Abstract—Different RNA fractions, as well as DNA disappeared from the endosperm of Opaque-2 maize 31 days after pollination. At the same time, these nucleic acids were present in the embryo. As compared with normal maize, Opaque-2 endosperm showed high RNase activity, which increased about ten-fold 31 days after pollination. Most of the activity was due to RNase A during the early stages. The disappearance of nucleic acids from the endosperm at a critical stage might be responsible for depressed zein synthesis in Opaque-2, since little of this protein is synthesised in the earlier stages after pollination.

INTRODUCTION

THE ETHANOL soluble zein fraction of proteins of maize grains is extremely deficient in lysine and tryptophan and the higher concentration of these amino acids in Opaque-2 maize is a direct result of depressed zein synthesis.[1] Higher ribonuclease activities in whole grains and endosperms of developing grains of Opaque-2 as compared with those in normal maize, have been reported.[2–4] In this paper, in an attempt to understand the possible mechanism of inhibition of zein synthesis, the changes taking place in the nucleic acid and protein fractions in the endosperm and embryo of Opaque-2 grains at different stages of maturity have been studied. Variations in the activities of RNase A and B in endosperm and embryo of both normal and opaque maize were examined with the object of understanding the degradation of RNA during grain development. It is shown that both RNA and DNA completely disappear from the endosperm during the penultimate stages of grain maturation. Negligible quantities of zein are synthesized prior to that stage. Amino acid composition of the protein fractions at different post-pollination stages has been determined.

RESULTS

RNA present in the endosperm of the immature grains was greater 17 days after pollination and completely disappeared 31 days after pollination, while its concentration in the embryo increased by about 35% (Table 1). Ingle *et al.*[5] observed a decrease in RNA content of the endosperm in a normal maize variety during grain development but did not observe its complete disappearance. Moreover the onset of the decrease was from 28 days after pollination in their study. It is unlikely that the nucleic acids are hydrolysed by RNase during the extraction procedure, since under these conditions RNase activity was negligible.

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[4] C. M. WILSON and P. E. ALEXANDER, *Science* **155**, 1575 (1967).
[5] J. INGLE, D. BEITZ and R. H. HAGEMAN, *Plant Physiol.* **40**, 835 (1965).

TABLE 1. RIBONUCLEIC ACID CONTENT OF EMBRYO AND ENDOSPERM OF OPAQUE-2 (mg/g dry wt.)

Days after pollination	Embryo	Endosperm
17	8·90	5·93
24	9·84	3·46
31	11·74	nil
38	12·02	nil

The phenol-extracted nucleic acids were fractionated on a methylated albumin kieselguhr (MAK) column. Soluble RNA (*s*RNA) was eluted in one main peak shouldered by a minor one. In the embryo, the DNA eluted mostly in a sharp peak shouldered by a minor one, but in the endosperm the DNA peak was broader. Ribosomal RNA (*r*RNA) showed two peaks representing light and heavy components. The latter was shouldered by another peak which was messenger RNA. About 95–98% of the total nucleic acids loaded on the column were recovered. The extraction procedure was reproducible and was repeated till no further nucleic acids were extracted. The individual peaks within any one elution profile on a MAK column could be directly compared with any other. From the percentage distribution of nucleic acids into *s*RNA and *r*RNA (Table 2) it was interesting to note that DNA

TABLE 2. FRACTIONATION OF NUCLEIC ACIDS FROM EMBRYO AND ENDOSPERM OF OPAQUE-2 ON MAK COLUMN

		Nucleic acid fraction as % of total		
	Days after pollination	Soluble RNA region	DNA region	Ribosomal RNA region
Whole seed	10	21·6	11·3	67·1
	17	9·0	14·4	76·6
	24	8·3	16·1	75·1
Embryo	31	7·5	11·0	81·5
	38	7·4	10·6	82·0
	17	21·9	10·4	67·7
	24	23·2	14·9	61·9
Endosperm	31	nil	nil	nil
	38	nil	nil	nil

also disappeared from the endosperm during the last stages of maturation. The proportion of *s*RNA was much higher in the endosperm than in the embryo. In the embryo *r*RNA constituted about 75–80% of total nucleic acids and the proportion of DNA decreased only slightly during grain development.

The disappearance of RNA from the endosperm coincided with enhanced RNase activity (A+B) which increased ten-fold 31 days after pollination in Opaque-2 maize endosperm (Table 3). RNase activity per endosperm also increased four-fold between 24 and 31 days in Opaque-2. In normal maize the activity was much less. No significant increase in activity was observed in the embryo. From the relative proportions of activities at pH 6·0 and 5·2 it

TABLE 3. RIBONUCLEASE ACTIVITY (UNITS PER mg PROTEIN) IN EMBRYO AND ENDOSPERM OF OPAQUE-2 AT DIFFERENT STAGES OF DEVELOPMENT

Variety			Days after pollination 17	24	31
White Opaque-2	Embryo	RNase (A + B)	25·1	30·1	29·5
		RNase pH 6·0/5·2	0·63	0·72	0·77
	Endosperm	RNase (A + B)	38·9	46·0	403·0
		RNase/endosperm	233·4	331·0	1410
		RNase pH 6·0/5·2	0·63	0·68	0·72
Normal maize	Embryo	RNase (A + B)	29·5	34·5	35·8
		RNase pH 6·0/5·2	0·66	0·71	0·76
	Endosperm	RNase (A + B)	33·8	39·0	180·0
		RNase pH 6·0/5·2	0·66	0·67	0·70

appears that most of the activity is due to RNase A in the early stages but RNase B increased slightly in later stages. Dalby and Davies[2] however did not observe any increase in the ratios. It was also observed that during storage of immature seeds at 0–4° and at room temp. for 4 hr, the total activity increased 4- and 9-fold respectively (Table 4). This rise in

TABLE 4. EFFECT OF ADDITION OF BENTONITE AND STORAGE ON RIBONUCLEASE ACTIVITY IN 24 DAY ENDOSPERM

	Units per mg protein RNase (A + B)	RNase pH 6·0/5·2
Homogenate assayed immediately	39·0	0·67
Homogenate + bentonite addition	16·0	0·68
Kernels kept at 0° for 4 hr	128·0	0·95
Homogenate kept at 0° with bentonite for 4 hr	35·0	0·70
Kernels stored 4 hr at 22°	400·5	0·78

enzyme activity due to injury on detachment of kernels might be accounted for either by induced enzyme activity or by new enzyme synthesis. Similar increases in RNase activity on injury have been observed for wheat[6] and tobacco[7] leaves. According to Bagi and Farcas,[7] the rise in enzyme activity was inhibited by actinomycin-D or other protein and nucleic acid inhibitors. Hence the enzyme increase during injury might also involve new enzyme synthesis.

When bentonite at 240 μg/ml was added to the homogenate, 70% of the activity was removed.

[6] D. HADZIYEV, S. L. MEHTA and S. ZALIK, *Can. J. Biochem.* **47**, 273 (1969).
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TABLE 5. DISTRIBUTION OF PROTEIN IN ENDOSPERM AND EMBRYO IN OPAQUE-2 MAIZE AT DIFFERENT STAGES OF MATURITY

Days after pollination	mg/embryo	mg/endosperm
10	3·93/seed	
17	4·4	8·4
24	6·0	11·8
31	6·6	12·7

It was observed that accumulation of protein in the developing grain was very rapid up to 24 days after pollination (Table 5). However in the endosperm it stopped after this stage and it continued in the embryo at a slower rate. Fractionation of endosperm proteins showed that during ripening, the albumin fraction decreased with a corresponding increase in the

TABLE 6. PROTEIN FRACTIONATION IN OPAQUE-2 MAIZE ENDOSPERM

Days after pollination	Percentage distribution of protein in different fractions				
	Albumin	Globulin	Prolamine	Glutelin	Recovery
10 (whole seed)	75·91	6·48	9·27	8·32	49·66
17	62·16	5·88	5·04	26·92	91·04
24	51·45	5·71	4·89	37·93	88·09
31	30·28	9·26	9·10	53·26	72·75
38	28·80	13·10	14·46	43·73	87·46

glutelin fraction (Table 6). The zein (prolamine) fraction increased during the latter stages of maturation. However, even in the mature grains of Opaque-2 the albumin fraction was much larger than the prolamines. The zein was characterized by high glutamic acid and low lysine concentrations (Table 7). The amount of leucine in this fraction increased considerably during maturation. Albumin, globulin and glutelin fractions were richer in lysine than the prolamines.

TABLE 7. AMINO ACID COMPOSITION OF PROTEIN FRACTIONS OF DEVELOPING ENDOSPERM OF OPAQUE-2

Days after pollination	Protein fraction	Grams amino acid per 100 g protein			
		Glutamic acid	Isoleucine	Leucine	Lysine
17	Albumin	14·6	3·8	5·4	6·1
	Globulin	15·3	4·2	6·2	5·7
	Prolamine	22·4	3·8	12·8	1·2
	Glutelin	12·8	3·9	8·8	3·8
31	Albumin	18·3	3·7	5·4	5·1
	Globulin	16·1	3·6	6·1	5·8
	Prolamine	25·4	4·6	19·4	0·3
	Glutelin	14·6	4·5	10·8	4·6

DISCUSSION

Complete disappearance of both RNA and DNA from the endosperm at later stages of maturity appears to be responsible for lack of substantial synthesis of protein in the endosperm. Since very little zein is synthesized prior to that stage in Opaque-2, the net result is a depressed zein content in the mature seeds. As compared with normal maize the RNase activity in the endosperm of Opaque-2 is very high 31 days after pollination. Dalby and Cagampang[3] attribute this effect to high RNase accumulation in Opaque-2 prior to 16 days post-pollination. Our results also indicate about 50% higher specific activity at 17 and 24 days after pollination in Opaque-2 as compared with normal. However at 31 days it is more than 10-times higher compared to activity at 17 days. Zein is rich in glutamic acid and leucine and poorer in lysine, while the protein fractions synthesized in the early stages after pollination are relatively richer in lysine.[8] Experiments are now under way to compare the synthesis of zein in normal maize endosperms, particularly in the last stages of maturation. However, comparatively low RNase activity in these indicates that perhaps zein synthesis is not abruptly stopped as in the case of Opaque-2. In normal maize there is evidence to show that most of the zein is accumulated in the endosperm during the penultimate stages of grain maturation.[8]

EXPERIMENTAL

Opaque-2 maize developed at this Institute was grown in the farm in the summer of 1970. After pollination cobs were harvested after 10, 17, 24 and 31 days. The grains matured at 38 days. The endosperm and the embryo from each grain were separated, except in the 10 days post-pollination stage, where whole grains were used. The results reported are an average of at least duplicate experiments in which the values agreed very closely.

The tissues were macerated at 0° with twice the volume of 0·1 M Tris-HCl buffer (pH 9·0) containing 0·5% sodium dodecyl sulphate (SDS) and 250 μg/ml bentonite with an equal volume of cold 80% phenol. The homogenate was shaken for 1 hr and then centrifuged at 12,000 *g* for 20 min. The phenol layer was washed once with the buffer. Further phenol-SDS extraction did not yield any additional nucleic acids. The pooled supernatant was deproteinized twice with phenol in the presence of bentonite and the nucleic acids were pptd. with 0·1 vol. of 10% NaCl and 2·5 vol. of EtOH. The ppt was suspended in 0·05 M phosphate buffer pH 6·7 and further deproteinised by chloroform-amyl alcohol treatment.[9] The purified nucleic acids were dialysed against phosphate buffer at 4° for 8 hr.

Fractionation of nucleic acids. The nucleic acids were fractionated on a methylated albumin kieselguhr (MAK) column (10 × 2·5 cm), by slight modifications of the methods of Mandell and Hershey[10] as reported by Hadziyev *et al.*[11] Fractions (3 ml) were collected and the OD read at 260 nm. Individual nucleic acid fractions were separated and identified as described earlier.[6] The soluble RNA fraction had amino acid acceptor activity and the rapidly metabolizable RNA was found to shoulder ribosomal RNA in pulse labelling study using ^{32}P.[6]

Protein fractionation. The proteins were fractionated by the method of Nagy *et al.*[12] Since fresh material was used for protein extraction, defatting treatment was given after water extraction. Suitable aliquots were hydrolysed in 6 N HCl in sealed evacuated tubes. A Technicon Analyzer was used for amino acid determination.

Ribonuclease assay. The extraction and conditions for assay of ribonuclease activity at pH 5·2, 5·8 and 6·0 were the same as have been reported by Hadziyev *et al.*[6] and Wilson.[13] The results are expressed as enzyme units, one unit of RNase activity corresponding to the amount of enzyme which causes an increase of 0·1 in absorbance over an enzyme blank. Protein was determined according to the method of Lowry *et al.*[14]

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RNA was determined by the orcinol method.[15] Although this method is not very specific the values agreed with absorption at 260 nm. The DNA in the total nucleic acid fractions was determined by the area under DNA peak in elution profiles and these values were checked by DNA estimation using Burton's modified diphenylamine method.[16]

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Key Word Index—Zea mays; Gramineae; nucleic acids; proteins; changes during development; ribonucleases.