Phytochemistry, 1977, Vol. 16, pp 19-24 Pergamon Press. Printed in England.

PROTEIN SYNTHESIS AND CHANGES IN NUCLEIC ACIDS DURING GRAIN DEVELOPMENT OF *SORGHUM*

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(*Revised received* 27 *July* 1976)

Key Word Index—*Sorghum vulgare*; Gramineae; nucleic acid; lysine; leucine; incorporation; grain development

Abstract—Changes in DNA, RNA, nitrogen, nucleotide composition and *in vitro* incorporation of leucine/lysine by polysomes have been studied during sorghum grain development. Both DNA, RNA and protein content increased substantially during grain development. Although RNase activity increased, it did not affect RNA accumulation. Minor changes in the nucleotide composition of *r*RNA and *s*RNA were observed during grain development. *In vitro* incorporation of leucine and lysine by polysomes indicate qualitative change in the *m*RNA during later stages of grain development and the substantial accumulation of proteins during this period ultimately results in accumulation of proteins rich in leucine and poor in lysine.

INTRODUCTION

The poor nutritive quality of sorghum grain proteins is mainly due to extreme deficiency of lysine and tryptophan and excessive content of leucine. Fractionation of endosperm storage proteins indicated that prolamin and residue fractions, which account for 50 % of total proteins, are rich in leucine and deficient in lysine. Protein and nucleic acid metabolism studies in normal and opaque-2 maize[1] have shown that regulation of *m*RNA is important in suppression of zein synthesis. Besides, it is well established that proteins deposited in the endosperm during the late maturation stage are nutritionally poor [1, 2]. Basic studies pertaining to protein synthesis and its regulation in developing sorghum grain are lacking. In the present study an attempt has been made to study nucleic acid metabolism during grain development by feeding thymidine-[^{3}H], uridine-[^{3}H] and orthophosphate-[^{32}P]. Polysomes obtained from developing grains have also been examined for their capacity for protein synthesis *in vitro*.

RESULTS AND DISCUSSION

DNA, RNA and nitrogen contents of grains at different stages of grain development are shown in Table 1. Both DNA and RNA content per seed increased during maturity. The increase in RNA was about 6 fold compared to 1.5 fold increase in DNA on a per seed basis. Sorghum endosperm even at maturity contained a very high proportion of both DNA and RNA. The embryo contributed a minor proportion of total nucleic acids of the grain. However, both DNA and RNA content when expressed on a weight basis showed a decrease at maturity due to the increase in weight of the grain as a result of starch and protein deposition.

Fractionation of nucleic acids on a MAK column

Table 1. DNA, RNA and nitrogen content during grain development

Days after ear emergence	DNA		RNA		N	
	μg/Seed	mg/g	μg/Seed	mg/g	mg/Seed	mg/g
10	6.6	1.8	7.5	1.9	0·09	21·9
17	9.2	0.8	15.0	1.3	0.29	20.8
24	11.8	0.4	28.6	0.9	0.37	17.8
31	10.0	0.4	45.5	1.5	0.61	14.8
Endosperm	8.3	0.3	40.1	2.8	—	—
Embryo	1.6	3.2	3.8	5.9	—	—

At the 31 days stage endosperm and embryo were separated from the grains.

showed one main peak shouldered by another peak for *s*RNA at 10, 17 and 24 days, while at 31 days there were three peaks in this region. DNA was eluted in a sharp peak at maturity, while at 10 days, it had a broad peak. Ribosomal RNA (*r*RNA) showed two peaks representing light *r*RNA (*lr*RNA) and heavy *r*RNA (*hr*RNA). About 95–98% of the total nucleic acids loaded on the column were recovered. Per cent distribution of various nucleic acids shown in Table 2 indicate minor differences at different stages of grain development. The proportion of nucleic acids in the *s*RNA and DNA regions showed a decrease at maturity, while that of the *r*RNA region increased when compared to the proportion at the 10 day stage.

Table 2. Proportional distribution of nucleic acid on MAK column at different stages of grain development

Days after ear emergence	Nucleic acid fraction (% of total)		
	*s*RNA region	DNA region	*r*RNA region
10	24.5	13.8	61.8
17	19.2	8.5	72.2
24	20.7	12.9	66.4
31	20.2	10.3	69.5

RNase activity increased by two fold during the late maturation stage (Table 3). From the relative proportion of activities at pH 6 and 5.2 it appeared that there is a decrease in the proportion of RNase B and the activity at late maturity stages is solely due to RNase A. Although the increase in RNase activity towards the later stages of maturation is mostly due to the degradative RNase, it does not result in the decrease in RNA in endosperm, unlike maize where higher RNase activity has been

Table 3. Ribonuclease activity at different stages of grain development

Days after ear emergence	RNAase (A + B)		RNAase pH 6.0/5.2
	/mg protein	/g fr. wt	
10	6.4	120	0.84
15	4.9	110	0.77
20	9.8	172	0.71
25	13.7	208	0.65
31	13.0	222	0.66

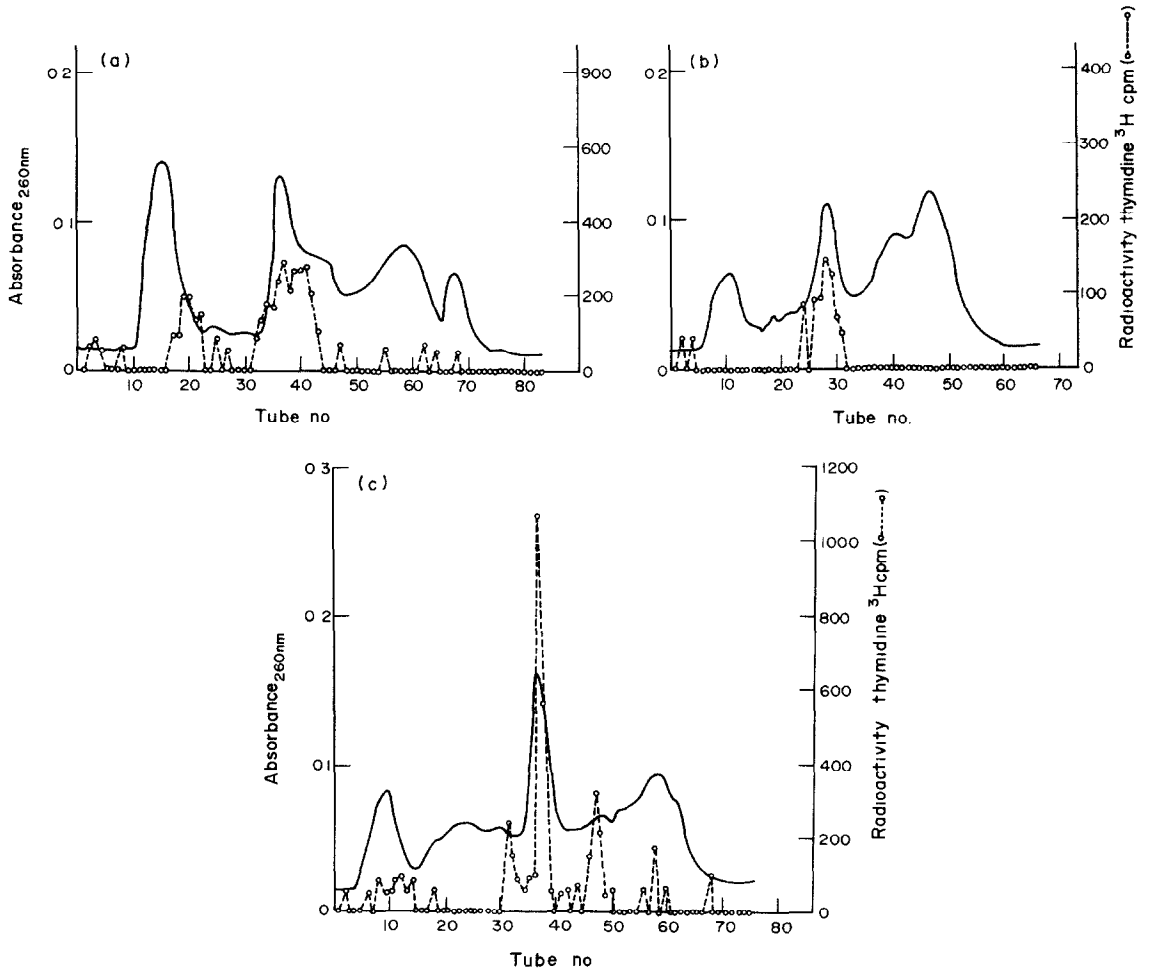

Fig. 1: MAK elution profiles of nucleic acids along with counts ^{3}H-thymidine at different stages of grain development. (a) At 10 days; (b) at 17 days; (c) at 24 days.

shown to be responsible for the disappearance or decrease of RNA towards maturity [1, 3]. In sorghum grain however the RNase activity is considerably lower than that in maize endosperm. Therefore, the substantial accumulation of nucleic acids in sorghum during later stages of maturity could be the result of considerably lower RNase activity and a lack of degradative function during grain development or to some mechanism of protection of RNA against RNase.

Thymidine-[^{3}H] incorporation

The absorption profile of nucleic acids on MAK column along with radioactive counts of thymidine-[^{3}H] are shown in Fig. 1. At early stages rapid labelling of DNA occurred. Traces of label also appeared in the *s*RNA region, which could perhaps be due to the presence of associated deoxyoligonucleotides. However, at 17 days after ear emergence, most of the label was found only in the DNA region. At the 24 days stage the DNA region had one major peak and a minor peak. The pattern of thymidine-[^{3}H] showed that DNA synthesis continued even at later stages of maturity.

Uridine-[^{3}H] incorporation

The absorption profile on MAK column along with radioactive counts are shown in Fig. 2. Very intense labelling in *s*RNA, light *r*RNA (1 *r*RNA) heavy *r*RNA (*hr*RNA) and *m*RNA was observed. Very little label appeared in the DNA region. At the 17 and 24 day stages the uridine incorporation was much less compared to the 10 days stage. At the 24 day stage incorporation was slightly more than that obtained at the 17 day stage. This showed that RNA synthesis was rapid at the 10 day stage compared to that at later stages of development. At later stages, RNA synthesis continued but perhaps at a slower rate.

Labelling of nucleic acids with orthophosphate-[^{32}P]

In order to know precisely the fate of rapidly metabolizable RNA, labelling with ^{32}P was studied in grains at 10, 17 and 24 days after ear emergence. Absorption profiles with the radioactive counts of individual fractions obtained by fractionation on MAK column are shown in Fig. 3. It was observed that at the 10 day stage, little

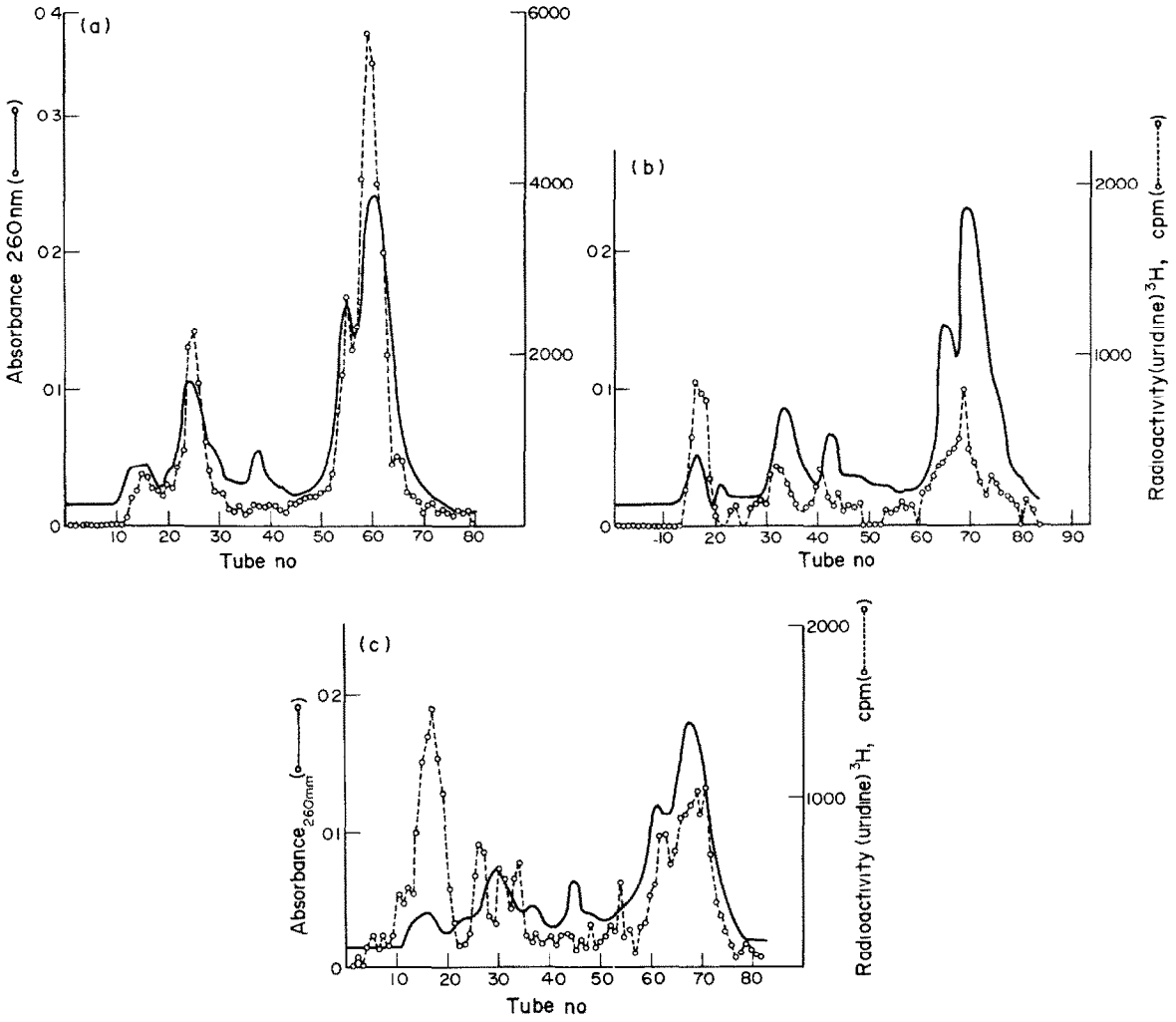

Fig. 2. MAK elution profiles of nucleic acids along with counts of ^{3}H-uridine at different stages of grain development. (a) At 10 days; (b) at 17 days; (c) at 24 days.

labelling occurred in DNA and *r*RNA region. Most of it was found in *s*RNA. However, at the 17 day and 24 day stages, very heavy labelling of *m*RNA region occurred. The *s*RNA and DNA region were also heavily labelled. The intense labelling at early stages in *s*RNA region may

Nucleotide composition

sRNA. On comparing the distribution of major nucleotides in *s*RNA it was observed that the guanylic acid (Gp) and cytidylic acid (Cp) contents were similar at 10, 17 and 24 days. Cp content was higher and Gp

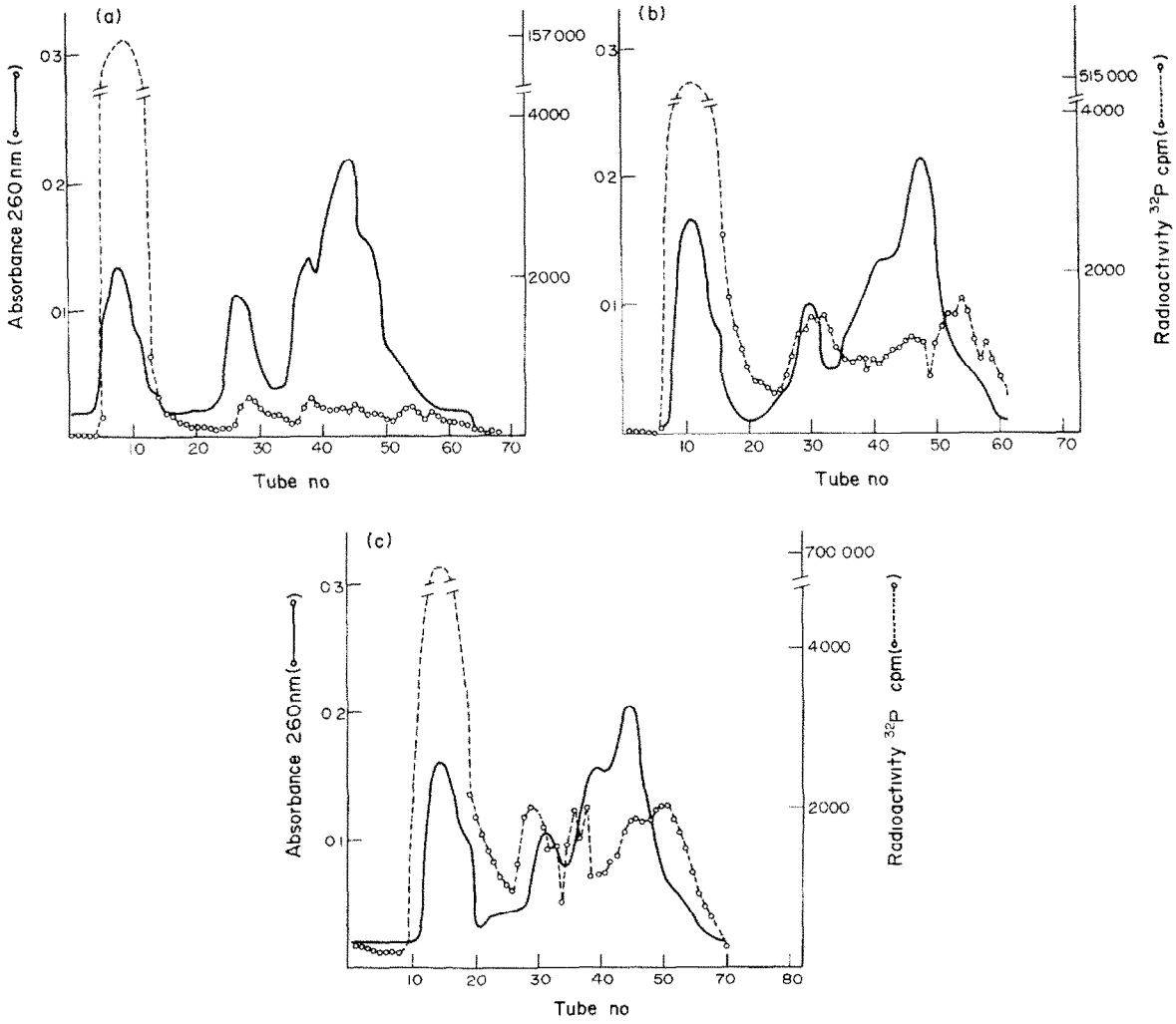

Fig. 3. MAK elution profiles of nucleic acids along with counts of ^{32}P-orthophosphate at different stages of grain development. (a) At 10 days; (b) at 17 days; (c) at 24 days.

perhaps be due to labelling of oligonucleotides which are rapidly metabolizable.

The labelling in the DNA region could arise from a fresh synthesis of DNA or from the formation of DNA *m*RNA hybrids or simply a complex of double stranded DNA, with *m*RNA strand wrapped around it. During these stages DNA synthesis was rapid and hence both these possibilities exist. The lesser extent of ^{32}P labelling at the 10 day stage could perhaps be due to a lower rate of nucleic acid synthesis at that stage. Rapid labelling at 17 and 24 days paralleled rapid protein accumulation in the grain. The sustained higher RNA levels at later stages of seed maturation and rapid labelling of nucleic acids with ^{32}P may be associated with the maintenance of the structural integrity of ribosomes and *r*RNA.

was lower at maturity (Table 4). Adenylic acid (Ap) content was lower at 17 days and slightly higher at the 24 day stage. Uridylic acid (Up) content also varied slightly during development. Concentration of pseudouridylic acid (ψUp) varied from 12.8 to 19.9 mol/100 mol of Up. The highest concentration was found at the 17 day stage. The values for ψUp in sorghum was higher than that obtained for wheat leaf total RNA by Hadziyev *et al.* [7].

The ratio of 6 amino/6-oxobases varied from 0.81–0.91 and the Ap/Cp ratio from 0.63–0.79. The sum of Gp + Up was always greater than the sum of Ap + Up (Type GC). The ratio of purine to pyrimidine nucleotides was lower than 1.0 and varied from 0.77 to 0.93. The changes in nucleotide composition during grain development reflect the changes in *s*RNA *in vivo*.

Table 4. Nucleotide composition of *s*RNA at different stages of grain development

Nucleotide (Mixture of 2′ and 3′ acids)	Days after ear emergence			
	10	17	24	31
	Mole percentage			
Cp	27.40	27.32	26.45	29.05
Ap	19.08	17.34	21.10	18.80
Up	22.38	23.63	21.30	24.21
ψUp	3.89	4.70	4.05	3.10
Gp	27.25	27.01	27.10	24.74
Purine/Pyrimidine				
(Ap+Gp)/Cp+Up+ψUp)	0.86	0.79	0.93	0.77
6 amino/6 oxobases				
(Ap+Cp)/Gp+Up+ψUp)	0.86	0.81	0.91	0.91
(Gp+Cp)/Ap+Up+ψUp)	1.20	1.19	1.15	1.16
Ap/Cp	0.69	0.63	0.79	0.64

Ribosomal RNA

On comparing the nucleotide composition of *r*RNA at different stages of grain development (Table 5) it was observed that the Cp content increased while the Ap content decreased during development. The Ap content was lower at maturity while the Gp content was higher.

Table 5. Nucleotide composition of *r*RNA at different stages of grain development

Nucleotide (Mixture of 2′ and 3′ acids)	Days after ear emergence			
	10	17	24	31
	Mole percentage			
Cp	21.60	22.29	23.50	27.00
Ap	28.60	24.50	24.28	17.00
Up	19.91	23.81	23.50	23.12
ψUp	1.00	0.88	1.18	0.80
Gp	28.89	28.51	27.46	32.08
Purine/Pyrimidine				
(Ap+Gp)/Cp+Up+ψUp)	1.35	1.12	1.09	0.96
6 amino/6 oxobases				
(Ap+Cp)/Gp+Up+ψUp)	1.00	0.87	0.91	0.78
(Gp+Cp)/Ap+Up+ψUp)	1.01	1.03	1.00	1.44
Ap/Cp	1.32	1.09	1.03	0.63

The sum of Gp + Cp was greater than the sum of Ap + Up (Type GC). This is in agreement with the observation of higher GC content in plant RNA [4–6]. ψUp was present in small amount to the extent of 3.45 mol/100 mol of Up at 31 days. This value is lower than the range of values obtained by other worker for *r*RNA from plants [7–9] and indicate purity of the preparation.

The ratio of purine/pyrimidine nucleotides, decreased during seed development. Since there was a marked accumulation of RNA, most of which was *r*RNA, during late maturity stage, the changing purine/pyrimidine ratios indicate that new *r*RNA formed has a nucleotide composition substantially different from that of early *r*RNA.

Characteristic of polysomes

Yield of polysomes was highest at 15 days stage. A total of five components representing ribosomal monomer, dimer, trimer, tetramer and pentamer were observed from the sedimentation pattern.

Amino acid incorporation in cell-free system

In order to study the influence of polysomes on the changed amino acid content of different protein fractions synthesized during grain development, the amino acid incorporation by polysomes was studied.

The effect of different co-factors in the incubation medium is shown in Table 6. Omission of ATP and GTP from the complete system reduced the incorporation by 86%. The incorporation was further reduced when creatine phosphate and creatine phosphokinase were omitted. Therefore, the incorporation is energy dependent. The polysome amino acid incorporation system was highly sensitive to RNase treatment and resulted in 96% inhibition. Similar inhibition by ribonuclease for various systems has been reported [6, 10].

Table 6. Characteristics of cell-free protein synthesizing preparation after 15 days of ear emergence

Incubation mixture	Incorporation of ^{14}C leucine cpm/assay tube after deducting background counts
Complete	1625
—ATP —CTP	225
Plus ribonuclease	125
—CPK —CP	160
—ATP —*Ribosomes*	142

Incorporation of leucine and lysine during *in vitro* protein synthesis is presented in Table 7. Incorporation of leucine increased slightly during grain development while that of lysine decreased markedly at the 20 day stage. On comparing the leucine/lysine ratio, it was observed that with grain maturation relatively lower lysine was incorporated. This resulted in higher leucine/lysine ratios. The reduction in lysine content was quite significant and this parallels the synthesis of protein fractions which are low in lysine and high in leucine. The results are in agreement with those obtained for maize polysomes [6].

Table 7. Incorporation of leucine vs lysine by isolated polysomes at different stages of grain development

Days after ear emergence	Yield of polysomes (mg/g fr. wt)	^{14}C-leucine incorporated cpm/mg RNA	^{14}C-lysine incorporated cpm/mg RNA	Lysine/leucine
10	0.59	4870	1890	0.39
15	0.77	4920	1670	0.34
20	0.69	5150	800	0.16

Leucine in protein at 10, 17 and 24 days after ear emergence was 8.48, 12.08, 13.79 g/100 g. protein respectively, while lysine in protein was 3.91, 3.13, 2.58 g/100 g protein respectively at these stages.

The results obtained in the present study indicate changes in the type of *m*RNA formed as evident from changed ratio of leucine/lysine incorporation during grain development. Even in maize endosperm it has been shown that the changing pattern of leucine/lysine

incorporation indicate that the regulation of *m*RNA is important in determining the zein accumulation. The ratios of leucine to lysine obtained in sorghum grain are higher in the present study compared to those obtained for maize endosperm [6] even at early stages of development. This is mainly due to higher rate of leucine incorporation and relatively low lysine incorporation by sorghum grain. Since substantial accumulation of protein occurs 17 days after ear emergence, the changing pattern of leucine/lysine incorporation thus results in accumulation of leucine rich and lysine poor proteins towards later stages of maturity. Therefore, the regulation of *m*RNA synthesis of degradation is important in determining protein quality.

EXPERIMENTAL

Sorghum variety CSH-2 was grown at I.A.R.I. farm. Cobs were harvested 10, 17, 24 and 31 days (Mature) after ear emergence. At 10 days seeds were taken together with the husk. Nucleic acids from the kernels at different stages were extracted purified and fractionated on MAK column according to the method used in ref [1].

Ribonuclease assay. The extraction and conditions for assay of ribonuclease at pH 5.2, 5.8 and 6 were the same as have been reported in refs [5] and [11]. One unit of RNase activity corresponding to the amount of enzyme which causes an increase of 0.1 in A over an enzyme blank. Protein was estimated by the method of ref [12]. RNA and DNA were estimated colorimetrically by the method of ref [13, 14].

Isolation of polysomes and amino acid incorporation. Polysomes from the kernels were isolated and purified according to the method of ref [6]. Amino acid incorporation was measured in the incubation mixture by the method of ref [6].

Nucleotide analysis of both *s*RNA and *r*RNA was done according to the method of ref [7].

Incorporation of uridine-[^{3}H] *and thymidine-*[^{3}H]. Basal parts of stalks bearing grains were dipped in a small beaker containing 20 uCi uridine-[^{3}H] (sp. act. 6400 mCi/m mol) or thymidine-[^{3}H] (sp. act. 3600 mCi/mmol) with small amount of H_2O and illuminated for 8 hr. Small quantities (1 ml) of H_2O were added 4 times to the beaker to ensure complete absorption. The seeds were removed, washed with cold 0.1 M uridine or thymidine and then with H_2O and stored in liquid N_2.

^{32}P-*labelling.* Basal parts of stalks bearing grains were dipped in a small beaker containing 1 mCi of ^{32}Pi with a small amount of H_2O and illuminated for 4 hr. The incorporation was stopped by washing the seeds 6 times with 50 ml portion of 0.1 M Pi, then with H_2O. Nucleic acids from uridine-[^{3}H] and ^{32}P treated seeds were isolated and fractionated as described above. In case of thymidine-[^{3}H] the following procedure was followed.

DNA Extraction. Seeds were ground in buffer I (0.1 M NaCl; 0.025 M Tris pH 7.5 and 10 mM EDTA) and filtered. Insoluble residue was washed twice with the same buffer. Residue was dispersed in 1% SDS in 25 mM Tris pH 7.3, in a mini blender for 30 sec. The slurry was transferred to a conical flask, stirred for 60 min after the addition of an equal vol of H_2O sat PhOH containing 0.1 8-hydroxy quinoline. Then it was centrifuged at 10000 *g* for 10 min. DNA was precipitated from the aq phase by addition of 2 vol of 95% EtOH. The ppt. was allowed to settle for 60 min and dissolved in saline–citrate 15 mM Na citrate, 0.15 M NaCl pH 7). To the supernatant an equal vol of 2 M NaCl was added and it was kept at 3° for 18 hr. and then centrifuged at 10000 *g* for 20 min. DNA was precipitated again from the supernatant with 2 vol of EtOH. DNA was spooled on a glass rod and was redissolved in saline citrate. Deproteinization was done with chloroform–isoamyl alcohol (24.1). DNA was reprecipitated from the aqueous phase with 2 vol EtOH and dissolved in saline–citrate and fractionated on a MAK column. The counting of different fractions was done using Bray's[15] scintillator in the case of uridine-[^{3}H], thymidine-[^{3}H] and for ^{32}P as Cerenkov radiation counting.

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