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CHANGES IN PROTEIN FRACTIONS AND LEUCINE-[^{14}C] INCORPORATION DURING *SORGHUM* GRAIN DEVELOPMENT

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Key Word Index—*Sorghum vulgare*; Gramineae; protein fractions; leucine-[^{14}C] incorporation; amino acids; tannins; grain development.

Abstract—Incorporation of leucine and changes in different protein fractions have been studied during *Sorghum* grain development. Most of the label from the injected leucine-[^{14}C] was found in glutelin and residue fraction towards later stages of maturity. The label in albumin, globulin and prolamin decreased with a concomitant increase in label in glutelin and residue proteins. The concentration of lysine, aspartic acid and glycine decreased while that of leucine, proline, alanine, tyrosine, phenylalanine, and cystine increased during grain development. Increase in serine, methionine, valine and isoleucine was only marginal. The proportion of glutamic acid was high at all stages of grain development. Glutelin fraction resolved into two peaks on gel chromatography, only one of which with higher MW was labelled, while in albumin both the peaks were found to be labelled. Tannin content also increased during grain development.

INTRODUCTION

It is well established that a high leucine to isoleucine ratio [1] coupled with low lysine is mainly responsible for the poor nutritive quality of *Sorghum* grain protein. Moreover the presence of tannins reduces the digestibility of proteins [2]. Ever since the discovery of high lysine mutants in maize, [3] barley [4], and *Sorghum* [5] various workers have studied protein and nucleic acid metabolism in developing grains, in order to understand the constraints that determine protein quality and quantity. In maize it has been shown that poor nutritive quality is mainly due to the deposition of zein which is extremely deficient in lysine and tryptophan, during later stages of grain development. In opaque-2 the improvement in nutritive quality is due to depressed zein synthesis. It has been further shown that regulation of *m*RNA synthesis is important in determining the protein quality during grain development [6]. During *Sorghum* grain development considerable turnover of the albumin or globulin fraction has been observed [7]. In this paper incorporation of leucine-[^{14}C] into various protein fractions during grain development has been studied because this amino acid is predominent in *Sorghum*. In addition incorporation *in vitro* of leucine and lysine by isolated polysomes has been investigated.

RESULTS AND DISCUSSION

Distribution of label from leucine-[^{14}C] along with the proportion of different protein fractions is presented in Table 1. As reported earlier the proportion of albumin decreased considerably while that of prolamin, glutelin and residual fractions increased during grain development. Globulin fraction remained more or less constant.

A major proportion of leucine was incorporated into the residual and glutelin fractions and much less label appeared in globulin, prolamin and albumin fraction. Most of the leucine incorporation occurred up to 17

Table 1. Percentage distribution of leucine-[^{14}C] at different stages of grain development

Days after injection	N/Seed mg	label % in TCA Soluble	label % in TCA Insoluble		Albumin	Globulin	Prolamin	Glutelin	Residue
	0.29	9.46	90.53	label %	10.9	5.51	5.27	26.8	51.6
17		(29.6)	(70.4)	cpm/mgN	1180	258.0	2270	7480	7760
				% of total protein	36.9	10.7	9.18	14.5	28.6
	0.37	9.68	90.31	label %	11.3	7.84	5.79	29.6	45.5
24		(25.9)	(74.1)	cpm/mgN	1380	2000	1310	5340	4550
				% of total protein	25.8	11.8	13.70	17.4	31.1
	0.61	7.02	92.85	label %	5.50	2.48	4.55	33.0	54.4
31		(10.5)	(89.5)	cpm/mgN	1520	618	879	4650	5430
				% of total protein	13.0	10.9	17.1	25.5	33.6

( ) represents %N of total N.

days after ear emergence and very little later on. Concentration of leucine-[^{14}C]/mg N in individual protein fractions during grain maturation progressively decreased in almost all fractions. At maturity, the proportion in the residue protein was much higher. Although the proportion of globulin remained more or less constant, the proportion of label decreased at later stages of maturity.

From the proportion of counts in TCA soluble and TCA insoluble fractions it was found that 90.5% of the leucine-[^{14}C] was incorporated by 17 days into proteins, and at maturity 93% label was in protein and remaining in the free pool. The label in the free amino acid pool was lesser during later stages of grain development. During this period substantial accumulation of protein occurred in the grain. In addition the proportion of label in the albumin fraction also decreased considerably during later stages of grain development. The decrease in the label in albumin and globulin fraction paralleled the increase in glutelin and residue fractions. The label in glutelin and residue fraction taken together accounted for *ca* 78% although in amount these fractions constitute only 43% of the total protein at the 17 day stage. This showed a very rapid incorporation of leucine in glutelin and residue proteins. Towards later stages of maturity, i.e. from 24 days after emergence to maturity the increase in label in leucine almost paralleled the increase in the amount of these fractions. The disappearance of label in albumin and globulin fraction and its appearance in glutelin and residue fraction towards maturity suggest either a turnover of proteins or its incorporation into glutelin and residue fractions. Since the amino acid composition of albumin and globulin fraction is grossly different from that of glutelin and residue proteins it would necessitate, modifications in these proteins for conversion to glutelin and residue proteins. Using ^{15}N urea and ammonium sulphate it has been observed earlier that albumin and globulin nitrogen appears in glutelin and residue protein fraction towards later stages of maturity [7]. The results obtained here further support this hypothesis.

In maize modification in various proteins during endosperm development has been observed which includes cleavage of lysine rich regions during later stages of maturity due to the action of specific protease [8]. It is likely that in *Sorghum* grains also, modifications by specific proteases may be involved.

Amino acid composition

The results presented in Table 2 show the amino acid composition of grain proteins at different stages of maturity. The decrease in nitrogen % during grain development is due to the faster rate of carbohydrate déposition compared to protein. The proportion of glutamic acid was the highest at all stages of development and remained more or less constant. Lysine decreased with grain development and at maturity it decreased by 40% compared to that at the 10 day stage. The proportion of leucine increased by 1.7 fold during grain development. The ratio of leucine/isoleucine increased from 2.14 to 3.17 during grain development.

Aspartic acid and glycine decreased while proline, alanine, tyrosine, phenylalanine and cystine increased during grain development. Serine and isoleucine increased marginally. Other amino acids, viz. methionine, valine, threonine and histidine did not follow a definite pattern.

Table 2. Amino acid composition at different stages of grain development (expressed on g/16 gN)

	10	17	24	31
Aspartic	11.21	10.07	8.49	7.19
Threonine	3.81	3.96	3.66	3.42
Serine	5.23	4.64	4.64	4.45
Glutamic	23.09	21.74	22.72	22.62
Proline	6.25	7.85	8.81	9.42
Glycine	4.39	4.10	3.51	3.28
Alanine	9.44	9.78	10.13	10.29
Valine	5.76	6.12	5.81	5.67
Isoleucine	3.96	4.54	4.57	4.58
Leucine	8.48	12.08	13.79	14.54
Tyrosine	3.27	4.07	4.58	4.70
Phenylalanine	4.22	5.19	5.65	5.71
Lysine	3.91	3.13	2.58	2.35
Histidine	2.06	2.02	1.99	2.34
Ammonia	3.06	2.62	2.77	2.77
Arginine	4.43	4.39	3.52	3.80
Methionine	1.87	1.74	1.79	1.80
Cystine	1.22	1.50	1.54	1.71
Tryptophan	1.23	1.22	1.20	1.10
%N	2.10	2.08	1.78	1.48
Leucine/Isoleucine	2.14	2.66	3.01	3.17

The amino acid data further show that the protein quality of the immature grain is superior to that of the mature grain. Deyoe *et al.* [9] reported higher levels of lysine, aspartic acid and glycine and lower levels of proline and leucine in immature grains compared to the mature *Sorghum* grains.

The changing pattern of amino acid composition in the grain is mainly due to the synthesis of different protein fractions at different stages of grain maturity. It has been shown earlier that prolamin, glutelin and residue proteins increase markedly during later stages. Since these fractions are deficient in lysine and rich in leucine, the overall result is a decrease in lysine content and increase in leucine content during grain maturation.

Besides in *in vitro* protein synthesis study increased incorporation of leucine during grain development and decreased lysine incorporation has been observed [10]. Since substantial protein synthesis occurs in *Sorghum* grain during last stages of grain development the changing pattern of leucine/lysine incorporation during this period results in lower lysine and higher leucine content.

Gel chromatography of leucine-[^{14}C] labelled protein

Albumin and glutelin fractions were fractionated on Sephadex G-100 and the elution pattern together with the label in different fractions is shown in Fig. 1. The relative elution volume (*Ve/Vt*) is shown in Table 3. In general, elution patterns of albumin and glutelin from immature grain were found to be similar to that of mature grain. The decrease in *Ve/Vt* of albumin fraction from mature grain indicates that it consists of larger MW components compared to immature grain. Minor differences also existed in the relative elution volume of the glutelin fraction. No radioactivity was found in the low MW fractions. In the case of albumin almost all fractions had radioactivity while in the case of glutelin only the

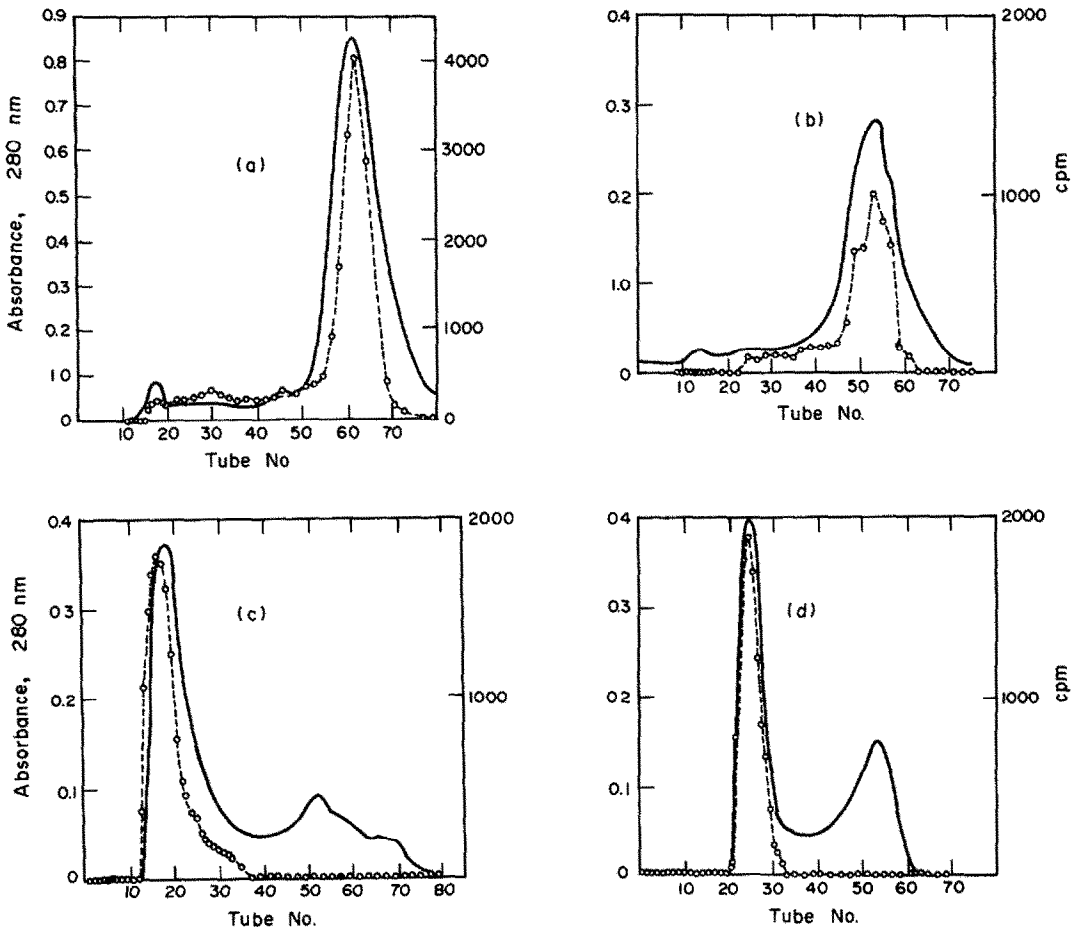

Fig. 1. Gel chromatography of crude protein fractions (albumin, glutelin) with radioactive counts. (a) Albumin at 17 days; (b) albumin at 31 days; (c) glutelin at 17 days; (d) glutelin at 31 days.

larger MW fraction (*Ve/Vt* 0.25, 0.32) showed radioactivity. This indicated that some fractions of glutelin have a very low leucine content. These investigations thus showed that the glutelin fraction is heterogenous and different molecular species in this fraction may have widely varying amino acid composition.

Tannin

The changes in tannin contents at different stages of grain development are shown in Table 4. The amount of tannin in grain increased during development and even at 17 days after ear emergence the grains had an appreciable concentration of tannins. Although the difference in tannic acid concentration when expressed on the basis of percentage sample was only 27%, on a per seed basis the increase was 3.5 fold. This difference is due to the increase in the wt of kernel during development. It is known that tannin protein binding reduce the solubility of the proteins. Hence, the increase in tannins might be contributing to the increase in residue proteins.

It is therefore evident that considerable changes occur in various protein fractions during grain development. Regulation of *m*RNA synthesis is perhaps involved in the changing pattern of protein accumulation. Besides, leucine incorporations *in vivo* indicates protein turnover especially during later stages of grain development.

Table 3. Gel chromatography of albumin and glutelin during grain development

Days after ear emergence	Fraction no.	*Ve/Vt*	
		Albumin	Glutelin
17	(i)	0.24	0.25
	(ii)	0.84	0.70
	(iii)	—	0.93
31	(i)	0.16	0.32
	(ii)	0.71	0.72

Ve = Elution volume *Vt* = Bed volume.

Table 4. % Tannin at different stages of grain development

Days after ear emergence	% Tannin (equivalent of tannic acid)	Tannins/seed (mg) equivalent of tannic acid
17	0.40	0.04
24	0.46	0.11
31	0.51	0.14

The amino acid composition of proteins at different stages further supports this conclusion.

EXPERIMENTAL

Sorghum variety CSH-2 was grown at IARI farm. The cobs were harvested at 10, 17, 24 and 31 days (Mature) after ear emergence.

Incorporation of leucine-[^{14}C]. At 10 days after ear emergence plants were injected with leucine-[^{14}C] (U) (8 μCi/plant, sp. act. 144 mCi/mmol) into the shank portion immediately below the ear at 2 diametrically opposite points. The wounds were sealed immediately with Quickfix. Samples were collected at weekly intervals after injection up to 3 weeks when the grains were fully matured. Kernels were harvested, separated and lyophilized.

Protein fractionation. Proteins were extracted by the modified Mendel–Osborne solvent extraction method as described in ref. [11]. The supernatant from the last extraction was checked for completeness of extraction. N was estimated by micro-Kjeldahl method [12]. Protein and non-protein fractions were separated by precipitation with 2.5% TCA as described in ref. [13].

Preparation of samples for counting TCA-soluble and TCA insoluble protein. After separating TCA insoluble and TCA soluble fraction, the ppt. was collected on glass fibre disk (Whatman GF/C) and washed 2 to 3 times with 2.5% TCA. Disks were transferred to scintillation vials, 10 ml of dioxane based scintillator [14] added and counted.

Counting of different fractions. Different fractions (5 ml) were concentrated to 2 ml and counted using a dioxane based scintillator. Material left after extraction (residue) was finally powdered and counted as a homogeneous gel in 10 ml of Bray's scintillation fluid and 4% CAB-O-Sil.

Amino acid analysis. Grains at 10, 17, 24 and 31 days were hydrolysed by conventional methods with 6N HCl. Amino acid analysis was done on an amino acid analyser. Results were calculated on 100% recovery. Tryptophan was analysed separately.

Tannin estimation. Tannin contents were estimated by Folin Denis method as modified in ref. [15].

Gel chromatography. Sephadex G-100 was allowed to swell in excess of H_2O. The slurry was deaerated and packed in a glass column (39 × 1.7 cm) maintained at 4°. The column was equilibrated by passing 0.2% NaOH and 5% NaCl in the case of glutelin and albumin respectively Protein fractions were layered on separated columns and the glutelin and albumin fractions were eluted with 0.2% NaOH and 5% NaCl respectively. The effluent was monitored at 280 nm and 4 ml fractions were collected; flow rate was maintained at 0.33 ml/min The fractions were counted for radio-activity using Bray scintillator.

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