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PROTEIN, NUCLEIC ACIDS AND ENZYME LEVELS DURING DEVELOPMENT IN A HIGH LYSINE SORGHUM GRAIN

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Key Word Index—*Sorghum vulgare*: Gramineae; sorghum; high lysine; protein; nucleic acids; enzymes; development.

Abstract—Dry wt, protein, free amino-N, RNA, DNA and the levels of hydrolytic enzymes have been measured in high lysine and normal sorghum grain during development. Dry wt was higher in CSV-5 throughout development. Protein accumulation/grain was lower in the later stages of development of IS 11758 than in CSV-5. The lower rate of protein accumulation in IS 11758 is not due to a limitation of free amino-N. CSV-5 had a higher proportion of prolamine. A major part of the prolamine in CSV-5 was deposited in a relatively short period from day 24 to 31. In this period much less prolamine was synthesized in IS 11758. RNA content was higher in IS 11758. DNA content, however, was higher in CSV-5 than IS 11758 during early stages of development. RNase activity at maturity was lower in IS 11758. Amylase activity/grain in both was similar, however, on a fr. wt basis it was higher in CSV-5. Protease level/grain was higher in IS 11758.

INTRODUCTION

Cereal grains are nutritionally poor because they contain low levels of lysine and/or tryptophan. In sorghum, a high level of leucine is responsible for the wide spread prevalence of pellagra in populations subsisting on it [1, 2]. Since the discovery of the association of high levels of lysine and tryptophan with the opaque-2 gene in maize [3], efforts have been made to obtain high lysine mutants of other cereal grains. As a result, high lysine genotypes of barley [4, 5] and sorghum [6, 7] are now available. However, like opaque-2 maize, high lysine sorghum has poor kernel characteristics and a lowered grain yield. The reduction in yield is mainly due to the shrunken nature of the kernel. Earlier studies [8, 9] have shown changes in protein fractions during development of normal sorghum grains. Biochemical studies carried out so far on high lysine opaque-2 maize [10, 11] and high lysine barley mutant Notch-2 [12], have shown that a reduction in the accumulation of starch is mainly responsible for decreased yields. It has also been shown that the decrease in starch and protein in opaque-2 endosperm is mainly due to reduced rate of protein and starch synthesis during the later stages of development [10, 11]. The constraints that limit the grain yield in high lysine sorghum are not fully known. In the present study, dry matter, protein accumulation, nucleic acid changes and changes in the activities of RNase, amylase and protease during grain development of high lysine and normal sorghum have been measured.

RESULTS

Dry wt, protein and free amino-N in developing grains of high lysine IS 11758 and normal sorghum CSV-5 are shown in Table 1. Dry wt/grain was substantially higher in CSV-5 than IS 11758 at days 17 and 31, and even at maturity the dry wt of IS 11758 grain was lower than CSV-5 grain. On comparison of dry matter accumulation/day by CSV-5 and IS 11758, it was found that CSV-5

Table 1. Dry wt, protein and free amino-N in normal (CSV-5) and high lysine (IS 1758) sorghum

Days after ear emergence	CSV-5			IS 11758		
	Dry wt (g/100 grains)	Protein (mg/grain)	Free amino-N (μg/grain)	Dry wt (g/100 grains)	Protein (mg/grain)	Free amino-N (μg/grain)
17	1.14	0.91	10.22	0.69	1.71	10.88
24	1.77	1.98	11.20	1.67	2.33	12.32
31	2.91	3.61	1.96	2.22	3.19	7.00
41	—	—	—	2.62	3.50	1.96

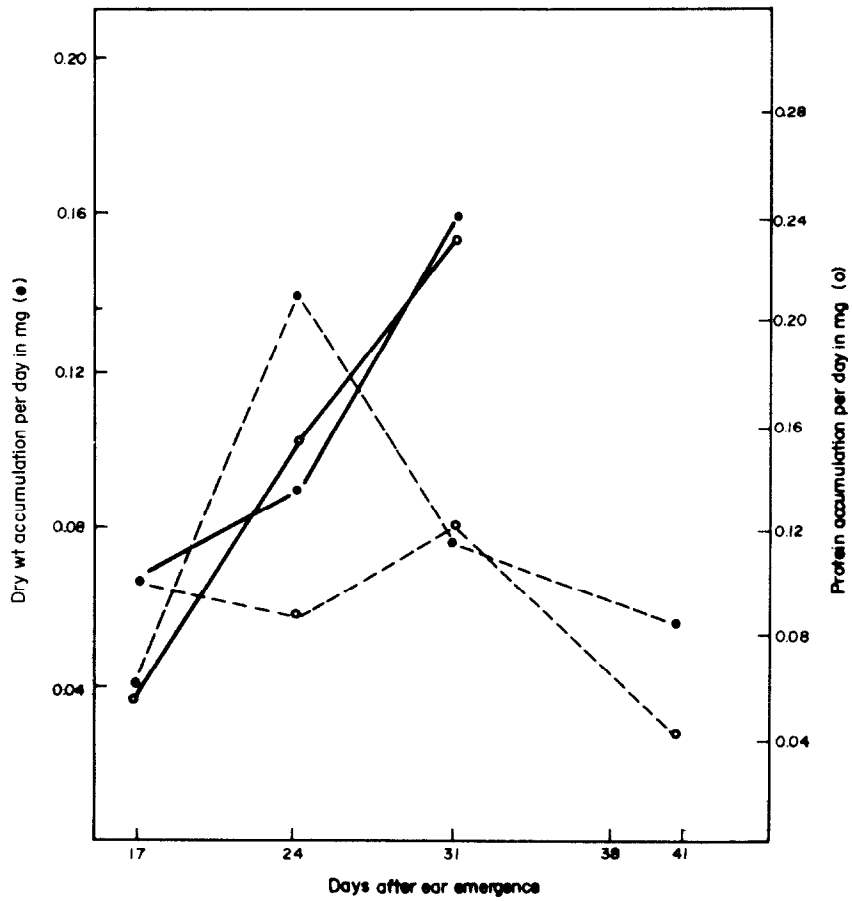

Fig. 1. Dry wt and protein accumulation day in developing grains of CSV-5 (——) and IS 11758 (– – – –).

accumulated more up to day 17, less between days 17 and 24 and more at day 31 (Fig. 1). Protein content/grain in early development was substantially higher in IS 11758 (Table 1). However, from day 24 to 31, CSV-5 grain accumulated 1.63 mg protein against 0.86 mg protein in IS 11758. Although high lysine grains matured in 41 days, the protein content/grain was not higher than CSV-5. Accumulation of protein/day/grain was substantially higher in CSV-5 after day 17 than IS 11758 (Fig. 1). Free amino-N/grain was nearly the same in both CSV-5 and IS 11758 at days 17 and 24. However, a marked decrease in free amino-N occurred at day 31 in CSV-5 and at days 31 and 41 in IS 11758. Despite the fact that up to day 24, the free amino-N was high in IS 11758, the rate of protein accumulation/day was still low from day 17 to 24.

The % distribution of protein fractions in developing grains of CSV-5 and IS 11758 is shown in Table 2. During grain development, a substantial decrease in albumin and an increase in prolamine I, prolamine II and glutelin occurred in both high lysine and normal sorghum. Globulin proportions did not change much in either variety. At maturity, high lysine sorghum had a higher proportion of albumin and glutelin and less prolamine than CSV-5. The increase in prolamines I and II from day 17 to maturity in CSV-5 was substantially higher than in IS 11758. To provide more information about the rate of synthesis of each protein fraction during kernel development, the protein fraction data have been expressed on a per grain basis (Table 3), since the % distribution of various fractions may be affected by an increase or decrease in one fraction only. In general, the albumin and globulin content/grain was higher in IS 11758 than CSV-5 at all stages of development. Prolamine I and II content was higher in IS 11758 up to day 24, but at maturity the amount of prolamine was substantially lower in IS 11758 than CSV-5. Normal sorghum CSV-5 grains deposited 78% of the prolamine present at maturity in IS 11758 grains in the 7 days from day 24 to 31. The glutelin fraction was higher in high lysine sorghum than normal sorghum at all stages of development, except day 31.

DNA and RNA contents in developing grains of IS 11758 and CSV-5 are shown in Table 4. DNA content/grain was considerably higher in CSV-5 during early development, whereas during later stages it was higher in IS 11758. RNA content showed a pattern different from DNA, as RNA/grain was considerably higher in IS 11758 than CSV-5 at all stages of development. At maturity RNA content in CSV-5 was 43% lower than IS 11758 grains.

RNase activity/grain in CSV-5 and IS 11758 (Table 5) did not differ much during development although the specific activities did show differences between the varieties. The differences in the specific activities of RNase

Table 2. Protein fractions in normal (CSV-5) and high lysine (IS 11758) sorghum during grain development

Variety	Days after ear emergence	% Total protein				
				Prolamine		
		Albumin	Globulin	I	II	Glutelin
CSV-5	17	33.33	10.48	9.05	11.90	16.19
	24	25.26	11.58	11.05	14.21	20.00
	31	11.54	9.89	13.74	23.23	25.27
IS-11758	17	32.08	9.17	9.17	15.00	22.08
	24	22.32	10.71	9.82	17.41	24.55
	31	16.59	11.98	11.16	18.43	26.73
	41	14.21	10.21	11.16	19.83	28.17

Table 3. Protein fractions in normal (CSV-5) and high lysine (IS 11758) sorghum during grain development

Variety	Days after ear emergence	Protein (mg/seed)				
				Prolamine		
		Albumin	Globulin	I	II	Glutelin
CSV-5	17	0.33	0.095	0.082	0.11	0.15
	24	0.50	0.23	0.22	0.28	0.40
	31	0.42	0.36	0.50	0.84	0.91
IS-11758	17	0.55	0.16	0.16	0.26	0.38
	24	0.52	0.25	0.23	0.40	0.57
	31	0.52	0.38	0.36	0.59	0.85
	41	0.50	0.36	0.39	0.69	0.99

Table 4. Nucleic acid changes in normal (CSV-5) and high lysine (IS 11758) sorghum during grain development

Days after ear emergence	CSV-5				IS 11758			
	DNA		RNA		DNA		RNA	
	(mg/g)	(μg/ grain)	(mg/g)	(μg/ grain)	(mg/g)	(μg/ grain)	(mg/g)	(μg/ grain)
17	0.75	9.87	4.24	38.2	0.28	3.33	5.40	46.0
24	0.63	14.00	2.50	45.4	0.46	11.22	3.25	82.2
31	0.38	10.55	3.25	90.3	0.63	14.00	—	—
41	—	—	—	—	0.50	11.90	7.57	102.3

Table 5. RNase activity in normal (CSV-5) and high lysine (IS 11758) sorghum during grain development

Variety	Days after ear emergence	RNase (A + B) (unit/mg protein)	(unit/g fr. wt)	(unit/seed)	pH 6.00/5.2
CSV-5	17	13.9	288.2	6.8	1.15
	24	25.2	393.2	12.6	1.17
	31	28.7	396.6	14.1	0.88
IS 11758	17	7.2	194.5	6.0	1.02
	24	13.9	331.7	14.0	1.08
	31	16.6	369.7	17.4	0.77
	41	17.8	346.1	9.7	0.55

Table 6. Protease activity in normal (CSV-5) and high lysine (IS 11758) sorghum during grain development

Days after ear emergence	CSV-5 (A^*/g)	CSV-5 (A/10 seeds)	CSV-5 (A/mg protein)	IS 11758 (A/g)	IS 11758 (A/10 seeds)	IS 11758 (A/mg protein)
17	2.29	0.54	0.083	4.17	1.30	0.12
24	1.39	0.45	0.060	2.01	0.81	0.07
31	1.16	0.44	0.053	1.45	0.65	0.05
41	—	—	—	1.09	0.30	0.044

* ΔA, absorbance at 280 nm.

Table 7. Amylase activity in normal (CSV-5) and high lysine (IS 11758) sorghum during grain development

Days after ear emergence	CSV-5 (A^*/g)	CSV-5 (A/seed)	IS 11758 (A/g)	IS 11758 (A/seed)
17	5.24	0.117	2.80	0.097
24	3.29	0.112	2.49	0.102
31	2.85	0.091	2.02	0.090
41	—	—	0.974	0.112

* A, absorbance at 550 nm.

are mainly due to the differences in the soluble protein content. RNase activity/g fr. wt was slightly higher in CSV-5 than IS 11758 grains. The ratio of RNase activity at pH 6.0/5.2 showed a decrease towards later stages of development. This indicates a relative increase in the degradative RNase. Protease activity/g fr. wt as well as the specific activities decreased during development in both CSV-5 and IS 11758 grains (Table 6). However, protease activity/g fr. wt was higher in IS 11758 than CSV-5 grains. Protease activity/grain was also higher in IS 11758 than in CSV-5. During development protease activity/grain decreased. The decrease in IS 11758 was greater than the decrease observed in CSV-5. However, at maturity the protease activity in IS 11758 was less than in CSV-5 grains. Amylase activity/g fr. wt varied during grain development (Table 7). Amylase activity/g fr. wt was lower in IS 11758 than in CSV-5 during development. The activity in IS 11758 was nearly half that of CSV-5 at day

17. Amylase activity/grain did not show much difference during development.

DISCUSSION

It is well established that the improvement in protein quality in high lysine mutants of maize [3], barley [4, 5] and sorghum [6, 7] is mainly due to the reduction in the proportion of prolamine together with an increase in albumins and glutelins. In maize, it has been suggested that the opaque-2 gene exerts a regulatory control on *m*-RNA synthesis for Zein formation at early stages of maturation [13]. In the present study, it has been found that normal sorghum grains accumulate two-fold more prolamine/grain from day 24 to 31, while in the case of high lysine sorghum, the increase was much less during this period. During development the glutelin content was also higher in IS 11758 than in CSV-5. Unlike high lysine opaque-2 maize the high lysine sorghum accumulated more RNA during the later stages of development. The increase in RNA/grain paralleled the increase in protein. Although on a unit weight basis the RNA content in high lysine sorghum grain was twice that found in CSV-5, the level/grain did not show much difference. The DNA content/grain increased during early development and showed a slight decrease at maturity.

The high level of soluble amino-N in early stages of grain development, its progressive decline as the rate of protein accumulation increased, and the very low level at maturity, indicates that the availability of free amino acids may control the grains' ability to synthesize protein, and that the lack of amino acids, rather than the loss of RNA, may limit protein synthesis. In spite of the extended period of development of the high lysine sorghum IS 11758, the total protein accumulated/grain up to maturity was still slightly lower than CSV-5. The slower rate of protein accumulation during later stages of grain development in IS 11758 compared to CSV-5 does not appear to be due to any limitations of free amino-N supply, as free amino-N content in both was nearly the same during grain development. RNase activity/grain was also substantially low in both sorghum grains compared to that reported in maize [14, 15].

It has been reported that the decrease in protein and yield in high lysine barley mutant Notch-2 as compared to parent NP-113 is not due to the activities of protease and amylase. The protease and amylase varied during development in both CSV-5 and IS 11758 but the levels of both enzymes were low compared to the values reported for high lysine barley grains [12]. Therefore, the reduced grain yield in high lysine sorghum IS 11758 is mainly due to the slow rate of dry matter accumulation during the later stages of grain development.

EXPERIMENTAL

Sorghum variety CSV-5 (normal) and high lysine (IS 11758) were grown in pots with uniform levels of fertilizer. Cobs were harvested at 17, 24, 31 and 41 days after ear emergence. CSV-5 matured in 31 days while IS 11758 matured at 41 days after ear emergence.

Protein fractionation. Protein fractionation was carried out according to ref. [16]. The supernatant from the final extraction was checked for completeness of extraction. N was estimated by micro-Kjeldahl.

Nucleic acids. RNA and DNA were estimated colorimetrically [17, 18].

RNase assay. The extraction and conditions for the assay of RNase at pH 5.2, 5.8 and 6 were as reported in refs. [19] and [20]. One unit of RNase activity was taken as the amount of enzyme which caused an increase of 0.1 in A over an enzyme blank.

Amylase activity. Total amylolytic activity was assayed by the method reported in ref. [21].

Protease activity. This was assayed according to ref. [22]. The enzyme was extracted with 0.05 M Tris containing 0.005 M cysteine (pH 7.5). The reaction mixture contained 1.0 ml 1% casein (substrate), 1.0 ml 50 mM Pi buffer (pH 7.5) and 0.25 ml enzyme. After incubation at 40° for 1 hr, 1.0 ml 20% TCA was added to stop the reaction. Tubes were kept for 2 hr at 0° for ageing and then centrifuged at 5000 g for 20 min. The $A_{280\,nm}$ of the supernatant was then measured. In the blank 1 ml 20% TCA was added at the start of the incubation period.

Soluble protein. This was estimated by the method of ref. [23].

The results reported are an average of independent duplicates, the values of which were always in close agreement.

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