Phytochemistry Vol 22 No 5 pp 1097 1101 1983
Printed in Great Britain

0031 9422/83/051097 05$03.00/0

BIOCHEMICAL CHANGES DURING SEED DEVELOPMENT IN SORGHUM (*SORGHUM BICOLOR*)*

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(*Revised received 11 October 1982*)

Key Word Index *Sorghum bicolor* Gramineae dry matter soluble sugars starch protein fat ash development

Abstract Grain dry weight accumulation soluble sugars starch protein fat and ash contents were studied in developing grains of eight sorghum cultivars The dry matter increased gradually from fertilization up to 35 days after flowering Variation in sugars and starch accumulation was observed for the cultivars The starch contents of high lysine mutant P 721 and high lysine lines IS 11167 and IS 11758 were comparatively lower and their protein contents were higher suggesting a possible mechanism for protein accumulation at the expense of starch accumulation RY 49 had a higher protein accumulation rate at various phases of maturation The fat content also tended to increase up to 28 days and the rate of accumulation was higher for RY 49 and IS 11167

INTRODUCTION

A better understanding of the pattern of accumulation and mechanisms by which various assimilates are accumulated in the grain after fertilization is important in any plant breeding program Further characterization of components in developing grain can help in identifying cereals with desirable composition and overall quality [1] High protein varieties of oat had greater ability during grain filling to remobilize nitrogen as compared to carbohydrate indicating genotypic variation for the capacity to accumulate chemical constituents [2] Increase of starch content with simultaneous decrease in free sugars in developing grains of two sorghum varieties has previously been reported [3] It is essential to determine the concentrations of the assimilates in the developing grain in order to understand the control mechanisms associated with metabolism during seed development Studies on the biochemical aspects of sorghum grain development are few This paper reports the changes that occur in sugars starch protein fat and ash contents during grain maturation of different sorghum genotypes

*Submitted as Journal Article No 249 by the International Crops Research Institute for the Semi Arid Tropics (ICRISAT)

RESULTS AND DISCUSSION

A description of the eight cultivars chosen for the study is given in Table 1 The cultivars represent a local type (M 35 1) an improved variety (CSV 3) hybrids (CSH 6 and CSH 8) high protein and high lysine shrivelled grain land races from Ethiopia (IS 11167 and IS 11758) a high lysine line from Purdue University U S A (P 721) and a high protein bold grain type (RY 49) from Ethiopia The concentrations of various constituents as percent of whole grain at different stages of maturation in the eight cultivars are given in Tables 2 and 3 The quantity present in mg grain of each constituent for five selected cultivars is given in Figs 1 6

Grain dry wt accumulation

The dry wt of grains increased gradually from fertilization up to 28 days for all the cultivars except IS 11167

Table 1 Basic description of the cultivars used in the study

Cultivars	Basic description and origin	Grain weight (g/100 seed)
M 35-1	A popular post rainy season cultivar in India	3 43
CSV 3	Variety released for rainy and post rainy seasons in India	2 47
CSH 6	Hybrid released for rainy season in India	3 25
CSH 8	Hybrid released for post rainy season in India	3 59
P 721	High lysine line from Purdue University U S A	2 35
IS-11167	High protein and lysine land races from Ethiopia shrivelled grain type	1 89
IS 11758		1 58
RY-49	High protein land race from Ethiopia plump and bold grain type	4 06

Table 2 Soluble sugars starch and protein contents in sorghum grain at different stages of development

	Days after 50 flowering																				
	Soluble sugars (%)							Starch ( )							Protein ( )						
Cultivars	7	14	21	28	35	42	49	7	14	21	28	35	42	49	7	14	21	28	35	42	49
M 35 1	8 9	5 5	3 3	1 4	1 2	1 1	0 9	55 7	67 9	73 3	73 8	73 5	72 2	74 5	13 6	9 2	7 1	6 7	6 8	7 6	6 8
CSV 3		4 5	1 4	1 4	1 3	1 4	1 3	43 0	70 1	73 3	71 8	72 4	71 2	73 4	16 8	10 1	8 9	9 5	8 5	9 7	7 9
CSH 6	8 6	4 8	2 5	1 3	1 0	1 1	1 3	62 5	68 5	70 7	70 3	70 0	67 7	68 0	11 0	10 4	8 0	9 2	7 7	8 7	9 5
CSH 8	9 9	4 3	2 3	1 6	1 8	1 3	1 3	58 9	67 9	71 9	72 1	72 2	72 9	73 1	12 0	8 2	6 3	6 9	7 0	6 7	7 2
P 721		7 2	6 5	3 2	5 1	3 3	2 0	56 8	65 2	65 9	67 2	63 7	64 7	64 1	13 6	12 1	11 3	11 6	13 2	12 1	12 0
IS-11167	14 4	8 8	10 7	7 9	6 0	4 8	5 5	53 4	58 7	57 1	56 6	57 2	55 4	55 9	16 8	16 9	17 8	18 5	17 8	19 5	19 6
IS-11758	9 9	9 9	4 7	3 6	3 8	4 4		60 1	58 5	56 9	55 6	57 0	55 3	—	17 2	18 2	18 3	19 2	18 0	19 1	
RY-49	3 4	2 7	2 4	2 1	1 9	2 0		66 0	67 9	69 9	69 1	70 8	69 7	—	14 9	14 2	13 8	14 9	14 1	14 7	

Table 3 Fat and ash contents in sorghum grain at different stages of development

	Days after 50 flowering													
	Fat ( )							Ash ( )						
Cultivars	7	14	21	28	35	42	49	7	14	21	28	35	42	49
M 35-1	18	20	28	28	29	28	25	28	18	15	16	16	15	15
CSV 3	21	31	31	33	31	32	29	33	18	16	16	17	18	18
CSH 6	16	26	31	31	33	34	31	19	18	14	13	13	13	13
CSH-8	18	23	26	26	30	30	29	24	17	14	13	13	14	15
P-721	20	20	31	37	36	36	33	21	18	18	17	19	17	17
IS 11167	32	55	63	66	63	68	67	28	25	28	27	27	28	30
IS 11758	53	62	61	56	59	57		25	28	28	28	29	27	
RY-49	22	27	31	36	38	36		21	20	18	19	18	19	

which declined after 21 days (Fig 1) Little change in dry wt was observed beyond 35 days Variation in the rate of dry matter accumulation existed among the cultivars Water loss may precede the completion of dry matter accumulation in sorghum [4] as reported for wheat and barley grains [5]

Soluble sugars and starch

The cultivars RY-49 CSH-8 CSV 3 and CSH-6 had lower soluble sugars than others between 7 and 14 days after flowering (Table 2) In general there was a reduction in the concentration of soluble sugars beyond 21 days and only little change was observed at later stages with the exception of P-721 which showed a decreasing trend towards maturity In RY-49 the percent sugar concentration remained fairly constant with development The high lysine Ethiopian lines (IS-11167 and IS-11758) produced high sugar concentrations throughout their development Wrinkleness of kernels in the Ethiopian lines may be associated with high sugar content as reported for maize [6] The quantity of soluble sugars per grain increased in all the cultivars up to 21 days with the exception of CSH 8 (Fig 2) A decline in the content of sugars was observed in the later phase of development This decrease in accumulation of sugars in the developing grain at later stages did not affect the rate of starch accumulation (Fig 3) Thus the observed changes in starch accumulation are unlikely to be attributable to the supply of sugars alone as reported in wheat [7]

Starch is the major component in sorghum which accounts for *ca* 43 66 of total dry matter even during 7 days after flowering in the cultivars studied (Table 2) The amount of starch as percent of sample on a dry wt basis increased slowly up to 21 days (Table 2) except for IS 11167 which showed a decrease Very little change was observed for starch concentration beyond 21 days This indicated that starch synthesis was completed during the very early stage of grain development in these lines (Fig 3) All the three high lysine types (P-721 IS-11167 and IS 11758) contained a comparatively smaller amount of starch both as percent of sample and as total quantity per grain (Table 2, Fig 3) The greater accumulation of sugars in these cultivars during the early stages of development and the smaller accumulation of starch indicate that starch synthesis was altered in these cultivars It has been proposed [8] that reduced starch synthesis in high lysine barley mutants may be due to an enzymatic deficiency in

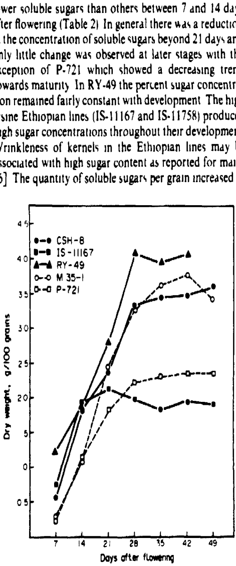

Fig. 1 Grain dry wt at different stages of maturation

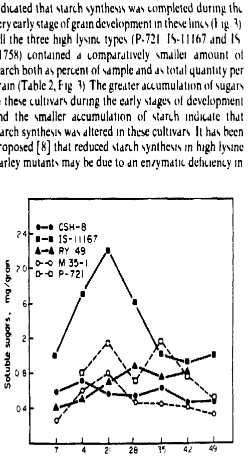

Fig 2 Soluble sugars content at different stages of grain maturation

the pathway of starch synthesis A similar mechanism may be present in the high lysine sorghum cultivars as well Sorghum lines with bold grains contained more starch unlike wheat [9] where kernel weight differences were largely attributed to constituents other than starch

Protein

The protein percentage decreased after 7 days for all the cultivars except in IS-11167 and IS-11758 (Table 2) There was a further slight increase in the two lines up to 28 days after flowering In other cultivars a decrease from 7 to 21 days was observed followed by little change thereafter (Table 2) This perhaps contrasts with the results of earlier workers [4 10] who noted that the protein percentage declines during later stages of grain development As a consequence of the protein increase in grain starch percentage was reduced in IS-11167 and IS-11758 which have a larger quantity of protein per grain The trend in protein accumulation per grain remained similar in all cultivars except RY-49 (Fig 4) where the rate of accumulation showed a steady increase up to 28 days after flowering Earlier workers [4] observed that the nitrogen was translocated into sorghum grains as long as the dry wt increased It was also evident that protein synthesis in the three Ethiopian lines may be much more efficient than in other cultivars because of different rates of protein accumulation (Fig 4) It was observed that CSH 6 which recorded 3 1 mg protein grain yielded 9 5 protein in the flour while IS-11167 with a similar protein content (3 7 mg grain) yielded 19 6 protein at maturity Similarly high protein oat varieties had a more efficient mechanism for the remobilization of nitrogen from the straw to the grain [11] Cultivars with low protein (e g

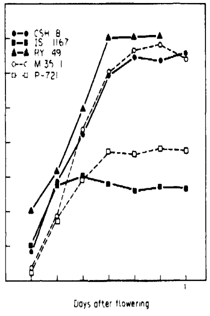

Fig 3 Starch content at different stages of grain maturation

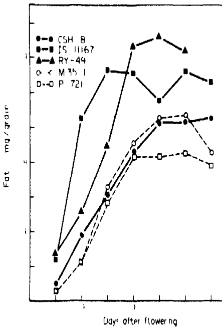

Fig 5 Fat content at different stages of grain maturation

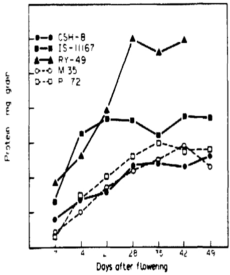

Fig 4 Protein content at different stages of grain maturation

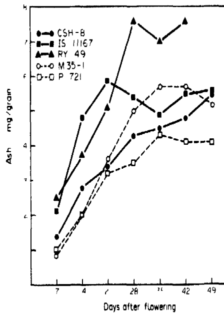

Fig 6 Ash content at different stages of grain maturation

CSH-8 and M.35-1) contain more starch (Table 2); the rate of starch accumulation was also high in these cultivars (Fig. 3). It is assumed [12] that any increase in the protein percentage of the grain will be associated with a proportionate decrease in the carbohydrate percentage. However, the quantities of starch and protein were higher in RY-49, indicating genotypic variation for starch and protein accumulation.

Fat and ash contents

The percentage of fat in the grains tends to increase slowly in all cultivars from 7 days to maturity (Table 3). Similarly the lipid content of wheat grain increased during maturation [13]. This increase may be associated with the development of aleurone tissues and other parts of endosperm containing a high level of lipids [14]. The rate of accumulation was rapid until 35 days after flowering and thereafter little change was observed (Fig. 5). The rate of accumulation was much greater in RY-49 and IS-11167 than in other cultivars. The increase in quantity per grain was similar to changes observed in dry matter accumulation. Lipid deposition is likely to be coupled with reduction in water flow into the grain during developmental stages [15].

The ash content, expressed as a percentage, showed some variation up to 21 days (Table 3), after which it remain unchanged. The quantity of ash per grain increased up to 35 days, except in IS-11167 and RY-49 which decreased from 21 and 28 days, respectively (Fig. 6). Beyond this stage the cultivars showed little variation for mineral contents with the exception of RY-49. Mineral accumulation was almost completed during the early phase of maturation (Fig. 6). Similar observations were observed earlier for sorghum [14], triticale, wheat and rye [16].

EXPERIMENTAL

Eight sorghum cultivars (Table 1) selected for the study were grown in the 1977 post-rainy season at ICRISAT Center, Patancheru (near Hyderabad), India. The genotypes were planted in plots arranged at random in three replications. Each plot consisted of eight rows each 6 m long. The rows were spaced 75 cm apart and plants within the rows were thinned to 11 cm spacing. A basal application of fertilizer (60 kg N/ha and 60 kg P_2O_5/ha) was included in the seed bed; a top dressing of 40 kg N/ha was given at 3 weeks after planting. Individual sorghum panicles were tagged at the 50% flowering stage; three panicles were harvested from each of the genotypes, at weekly intervals starting from day 7 after flowering until day 49. Grains were quickly separated from the whole panicles manually and freeze-dried. Equal weights of freeze-dried grains from each of the replicates were pooled and were ground in a Udy cyclone mill to pass through a 0.4 mm screen. The meal was defatted using *n*-hexane in a Soxhlet apparatus and used for further biochemical analysis. All analyses were carried out in duplicate.

Grain weight. Five replicates of each genotype containing 100 freeze-dried whole grains were weighed and the mean weight was determined.

Protein content. Total N in sorghum meal was determined by the micro-Kjeldahl method [17] and the crude protein was calculated (N × 6.25). The mean coefficient of variability for protein determination was 1.4%.

Soluble sugars and starch. Soluble sugars from sorghum meals were extracted with 80% EtOH in a Soxhlet apparatus. After evaporating the contents *in vacuo*, the residue was dissolved in H_2O and made up to a known vol. Total sugars were estimated by the PhOH-H_2SO_4 method [18]. Starch was estimated using the enzyme glucoamylase (Sigma) according to the procedure of ref. [19]. The mean coefficients of variability for soluble sugars and starch were 2.4 and 3.4%, respectively.

Fat and ash. Determined according to the method in ref [17]. The coefficients of variability for fat and ash determinations were 1.9 and 0.7%, respectively.

Acknowledgements We gratefully acknowledge the assistance of A. Laxma Reddy, T. Hanumantha Rao, P. Venkateswara Rao and K. A. R. Zafar.

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