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Nitrogen assimilation in opaque and normal sorghum during grain development

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Abstract. Activity of key nitrogen assimilating enzymes was studied in developing grains of high-lysine opaque sorghum P-721 and normal sorghum CSV-5. The higher percentage of protein in opaque sorghum was mainly due to lower starch content since protein per grain was less than in CSV-5. During grain development, albufn and globulin decreased while prolafne and glutelin increased. Prolafne content in CSV-5 was higher than in opaque sorghum. Average nitrate reductase activity in flag and long leaf were similar in both the varieties. The nitrate reductase activity decreased during grain development. Glutamate dehydrogenase activity was higher during early development and lower at later stages in opaque sorghum than in CSV-5. Glutamate oxaloacetate transaminase activity was higher and glutamine synthetase lower in opaque sorghum than in CSV-5 grains during development. Glutamate synthase activity was higher in opaque sorghum up to day 20 and lower thereafter than in CSV-5. It is suggested that reduced activities of glutamine synthetase as well as glutamate synthase in opaque sorghum as compared to CSV-5 during later stages of development may restrict protein accumulation in the former.

Keywords. *Sorghum vulgare;* Gramineae; high lysine; nitrate reductase; glutamate dehydrogenase; glutamate synthase; glutamine synthetase.

Introduction

Cereal proteins are nutritionally poor, mainly on account of deficiency of lysine and tryptophan. Although in sorghum, nutritionally superior genotypes have been identified (Singh and Axtell, 1973) or induced (Axtell *et al.,* 1979), these have not found acceptance due to lower grain yield.

Biochemical studies have shown that the reduction in the starch content of high-lysine barley and maize grain is mainly due to the decrease in one or two key enzymes of starch biosynthesis (Mehta *et al.,* 1979; Joshi *et al.,* 1980; Batra and Mehta, 198 1a, b). In high-lysine opaque-2 maize endosperm, premature termination of development results in a decreased yield of protein and starch, and consequently in a lower dry weight of the grain (Mehta *et al.,* 1979). The constraints that limit grain yield and protein content in high-lysine sorghum are not fully known. In this study we present a comparison of the key enzymes of nitrogen metabolism during grain development in high–lysine opaque P-721 and normal CSV-5 sorghum.

Materials and methods

Sorghum varieties CSV-5 (normal) and P-721 (opaque, high-lysine) were grown under uniform fertility conditions in IARI farm. The ears were harvested 15, 20, 25 and 30 days after emergence.

Abbreviations used: GDH, Glutamate dehydrogenase; GOT, glutamate oxaloacetate transaminase; GOGAT, glutamate synthase; GS, glutamine synthetase; NR, nitrate reductase.

Total nitrogen content of seeds after drying was estimated by micro-Kjeldahl method (AOAC, 1965), and the protein values obtained by multiplying by a factor of 6·25. Total free amino acids was estimated by the method of Lee and Takahshi (1966). Dry weight was estimated after oven drying at 100°C till constant weight was obtained.

Protein fractionation was carried out according to Landry and Moureaux (1970) as described by Misra *et al.* (1975). The completion of extraction for each protein fraction was checked by measuring absorbance at 280 nm in the last extractant before proceeding for extraction of the next protein fraction.

Enzyme assays

For assaying glutamate dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT), glutamate synthase (GOGAT) and glutamine synthetase (GS), seeds were ground in a chilled mortar in 50 mM Tris-HCl buffer (pH 7·5). The homogenate was centrifuged at 20,000 *g* for 15 min at 4°C and the supernatent used for enzyme assay. GDH was assayed by the method reported by Bulen (1956). GOGAT was estimated in the same manner as GDH except that 0·4 ml of 0·2 M glutamine was added instead of ammonium sulphate as amino donor in the reaction mixture. GOT and GS were assayed as described by Gupta *et al.* (1980) and Kanamori and Matsumoto (1972), respectively. Leaf nitrate reductase (NR) activity *in vivo* was assayed according to the method of Jaworski (1971) and Hageman and Flesher (1960). Soluble protein was estimated by the method of Lowry *et al.* (1951). The values reported are averages of triplicates in enzyme assays and duplicates in protein fractionation.

Results

Dry weight of CSV-5 grains was substantially higher than that of opaque P-721 grains at all the stages of development except at day 15 (table 1). Calculated as a percentage of the total dry weight of the grain, the protein content was higher in opaque than in normal CSV-5 sorghum. However, protein content/grain during develop-

Table 1. Dry weight, protein and free amino acids in normal (CSV-5) and high-lysine opaque (P-721) sorghum during grain development.

	CSV-5				P-721			
		Protein		Free amino acids		Protein		Free amino acids
Days after ear emergence	100-grain wt (g)	Dry wt (%)	mg/grain	µmol/ grain	100-grain wt (g)	Dry wt (%)	mg/grain	µmol/ grain
15	0·99 ± 0·04	13·6	1·35 ± 0·02	1·66	1·00 ± 0·02	16·2	1·62 ± 0·14	2·54
20	1·98 ± 0·02	12·9	2·55 ± 0·37	2·40	1·44 ± 0·02	15·4	2·22 ± 0·27	2·67
25	2·68 ± 0·07	12·0	3·22 ± 0·41	1·26	1·58 ± 0·02	14·9	2·36 ± 0·12	1·12
30	3·00 ± 0·03	11·4	3·41 ± 0·24	1·20	1·70 ± 0·05	13·9	2·37 ± 0·15	0·83

Values are mean ± SE

ment was higher in CSV-5 than in opaque P-721. The period of protein synthesis was longer in CSV-5 as protein continued to increase upto day 30, whereas in opaque P-721 very little protein was synthesized after day 20. Total free amino acid decreased from day 20 to day 30. Total free amino acids/grain was higher in opaque P-721 upto day 20 and lower thereafter than in CSV-5 grains.

Protein fractionations

The albumin + globulin fraction decreased and prolamine and glutelin fractions increased during development in both the varieties (table 2). At day 30, opaque P-721 grains had higher glutelin and lower prolamine fractions than CSV-5 grains. Data in table 2 on the rates of accumulation of individual protein fractions in developing grains show that more prolamine accumulated in CSV-5 than in opaque P-721. Prolamine/grain in CSV-5 at day 30 was almost twice that of opaque P-721. Glutelin/grain was the same in CSV-5 and opaque P-721 at later stages of development and albumin + globulin slightly higher in CSV-5.

Table 2. Protein fractions in normal (CSV-5) and high-lysine opaque (P-721) sorghum during grain development.

		Total protein (%)			
Variety	Days after ear emergence	Albumin + Globulin	Prolamine	Glutelin	Residue
CSV-5	15	43·4 (0·59)	21·5 (0·29)	16·0 (0·23)	11·0 (0·15)
	20	37·4 (0·96)	30·6 (0·78)	17·0 (0·43)	13·6 (0·34)
	25	29·2 (0·94)	34·9 (1·20)	18·7 (0·61)	14·6 (0·47)
	30	24·2 (0·84)	39·6 (1·38)	19·8 (0·69)	16·5 (0·58)
P-721	15	51·7 (0·85)	16·2 (0·28)	19·3 (0·32)	8·5 (0·14)
	20	37·4 (0·83)	24·4 (0·54)	24·4 (0·54)	11·0 (0·24)
	25	30·2 (0·71)	29·4 (0·69)	27·7 (0·65)	11·8 (0·28)
	30	24·7 (0·55)	31·4 (0·70)	30·5 (0·68)	13·4 (0·30)

Values i. parantheses represent mg per grain of protein fractions.

Enzyme activities

The two varieties had almost equal *in vivo* NR activity per g fresh weight or per leaf in the flag and long leaf averaged over the 30 day period (table 3), although the activity in each case fluctuated during development. In CSV-5, the activity peaked at day 20 and decreased later whereas P-721 showed a second increase towards day 30.

GDH activity per g fresh weight, specific activity and activity per grain in

Table 3. *In vivo* NR activity in leaves of normal (CSV-5) and high-lysine opaque (P- 721) sorghum during development.

		n mol NO_2^- formed/g fresh wt/30 min				
		Days after ear emergence				
Leaf	Variety	0	15	20	25	30
Flag	CSV-5	406 ± 9·0	318 ± 8·4	644 ± 8·7	591 ± 8·7	423 ± 26·3
	P-721	261 ± 3·7	680 ± 17·7	503 ± 26·3	318 ± 7·8	609 ± 15·3
Long	CSV-5	247 ± 9·0	212 ± 6·5	432 ± 7·8	371 ± 9·0	229 ± 17·7
	P-721	148 ± 2·3	335 ± 17·7	335 ± 17·7	212 ± 8·9	477 ± 15·3
		n mol NO_2^- formed/leaf/30 min				
Flag	CSV-5	513	1508	3090	2676	2250
	P-721	458	2800	1466	1857	2594
Long	CSV-5	1117	1867	3864	3022	2544
	P-721	828	3337	2686	2630	4282

developing grains followed nearly identical patterns (figure 1 A, B, C) in opaque P-721, peaking at day 20, being lowest at day 25 and increasing slightly again. Activity was lower in CSV-5 than in P-721 on days 15 and 20, and higher on day 30. GOT activity was higher in opaque P-721 than in CSV-5 upto day 25 (figure 1 D, E, F). The activity/grain was lower at day 30 in opaque P-721 than CSV-5. During development the activity increased upto day 25 and decreased at maturity in both varieties. GOGAT activity decreased from day 20 to maturity. Although activities in CSV-5 and opaque P-721 were different on all the days of assay, activity/grain averaged over development was nearly the same (figure 2A,B, C). Throughout development GS activity was higher in CSV-5 than in opaque P-721 (figure 2D, E, F). The activity in CSV-5 increased with development upto day 25 whereas in opaque P-721 the activity/g fresh weight as well as the specific activity were considerably low upto day 25 and showed a rapid increase at day 30.

Discussion

Earlier studies carried out with high-lysine opaque-2 maize (Mehta *et al.,* 1979) and high–lysine sorghum (Johari *et al.,* 1981) have shown a decrease in dry weight and protein in the high-lysine mutants as compared to normal grains. In the present study also protein content/grain was lower in high-lysine opaque sorghum P-721 than in the normal sorghum CSV-5. The lower protein content of opaque P-721 did not appear to be due to any limitation in the supply of free amino acids since the average total free amino acid content during development in opaque P-721 was similar to that in CSV-5. The decrease in protein content of opaque P-721 was mainly due to a decrease in the prolamine fraction at maturity, the level of prolamine in opaque P-721 at maturity was half of that in CSV-5 grains. Since prolamines are extremely deficient in lysine and tryptophan, the reduced rate of prolamine accumulation during later stages of grain development in opaque P-721 sorghum resulted in overall improvement of protein quality. In this respect it is similar to opaque-2 maize (Mertz *et al.,* 1964; Murphy and Dalby, 1971).

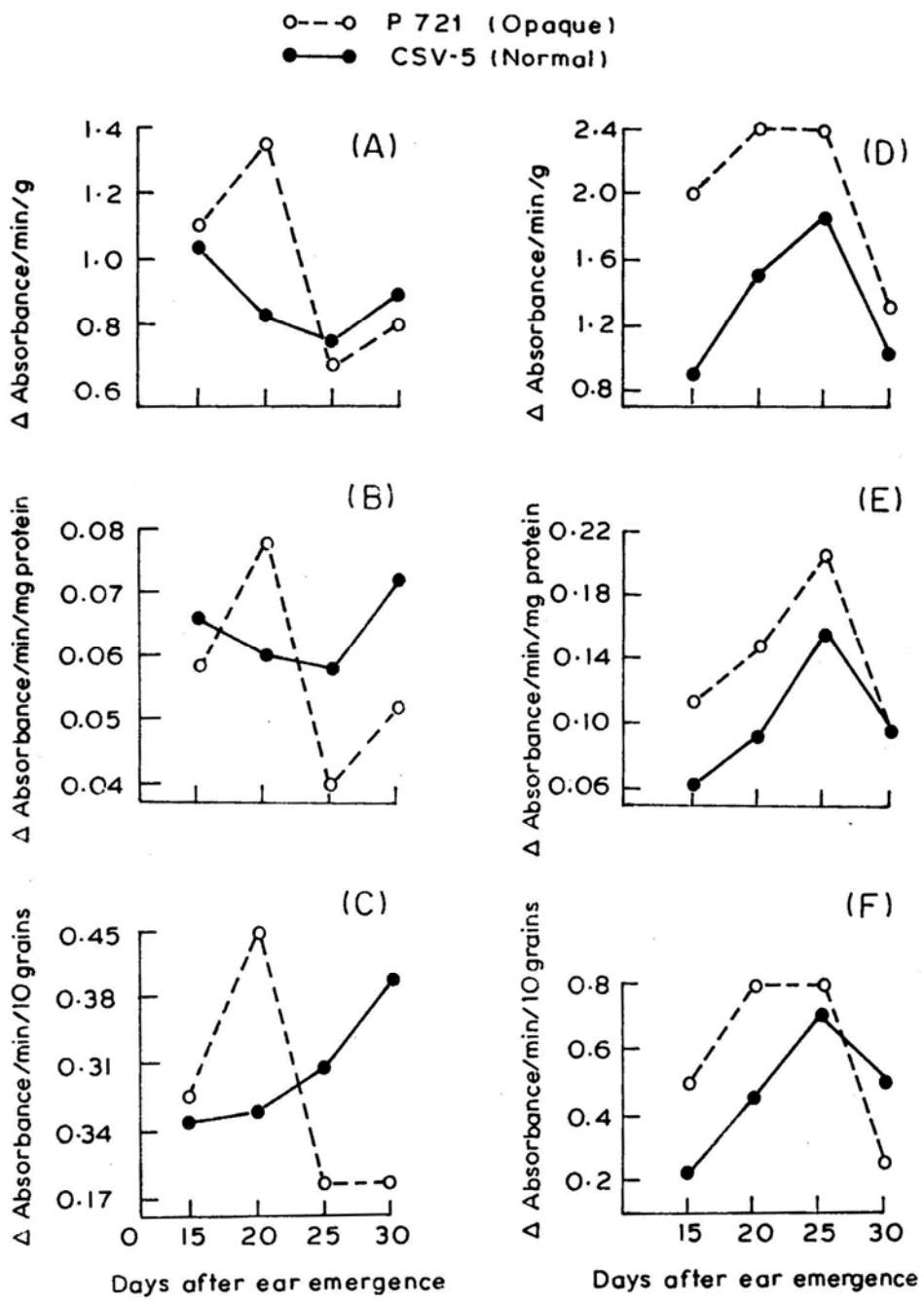

Figure 1. GDH activity: **(A),** per g afresh wt;. **(B),** per mg protein; **(C),** per 10 grains in normal CSV-5 (●) and opaque P-721 (– – O – –) sorghum grains during development.

GOT activity: **(D),** per g fresh wt; **(E**), per mg protein; **(F),** per 10 grains in normal CSV-5 and opaque P-721 sorghum grains during development.

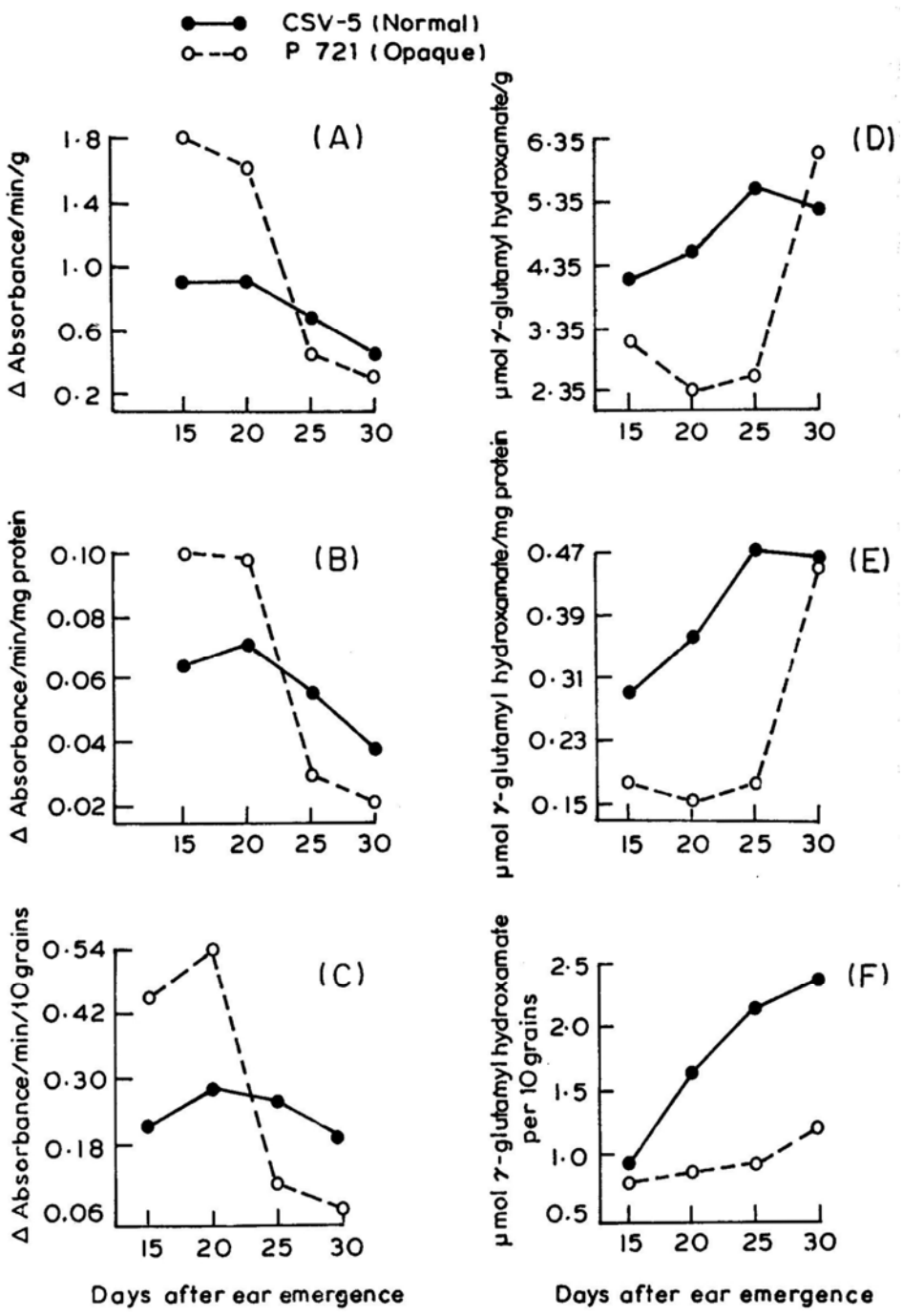

Figure 2. GOGAT activity; **(A),** per g fresh wt; **(B),** per mg protein; **(C),** per 10 grains in normal CSV-5 (●) and opaque P-721(. – – O — –) sorghum grains during development.

GS activity: **(D),** per g fresh wt; **(E),** per mg protein; **(F),** per 10 grains in normal CSV-5 and opaque P-721 sorghum grains during development.

The lower protein accumulation in opaque P-721 could be due to a lower rate of protein synthesis during later stages of grain development. It could also be due to lower activities of key nitrogen assimilating enzymes (GS and GOGAT) compared to activities in CSV-5. From day 20 to maturity the protein content of CSV-5 increased by over 30% while in opaque P-721 the increase was less than 10%. Although GOT activity was higher in opaque P-721, the reduced activity of GS as well as GOGAT during later stages of grain development may have a direct role in limiting protein synthesis. The lower activity of GS also coincides with the reduction in prolamine in high-lysine opaque P-721 sorghum grains. Tsai (1979) has also observed a decrease in GS activity and related the reduction in zein content of high-lysine opaque-2 maize endosperm. However, in normal maize endosperm, the increase in activities of GAGAT and GS coincided with increase in total protein during development (Sodek and Da Silva, 1977; Aaks *et al.,* 1979).

Nitrate assimilation rate as well as nitrate reduction capacity in flag and long leaf of opaque P-721 were similar to those of CSV-5. This rules out the possibility of constraints in nitrate assimilation and nitrate reduction in opaque P-721 grains *vis-a-vis* CSV-5.

Proteases have been implicated not only in protein turn-over but in post-translational modification of protein (Gupta *et al.,* 1977). Varying levels of activity of proteases have been reported (Curz *et al.,* 1970; Rao and Croy, 1972; Johari *et al.,* 1981). In the high-lysine barley mutant Notch-2 proteases do not play a role in decreasing protein content (Sen and Mehta, 1980). In sorghum opaque P-721, protease activity was in general, lower than in CSV-5, ruling out the possibility of the reduction in protein content resulting from degradation of protein (Chitra, 1981).

The results obtained therefore indicate that the decreased protein synthesis in opaque P-721 is not due to any constraint in nitrate assimilation but may be due to the reduced activity of GS and GOGAT towards the later stages of development. The depressed protein synthesis results in nutritional improvement since the protein deposited during later stages of development in normal CSV-5 sorghum grains consists of prolamine which is nutritionally poor.

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