AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C 20523
BIBLIOGRAPHIC INPUT SHEET

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Batch 42

1. SUBJECT CLASSIFICATION
A. PRIMARY: Serials — Y-AF30-0170-0000
B. SECONDARY: Food production and nutrition--Plant breeding--Cereals--Sorghum

2. TITLE AND SUBTITLE
Inheritance and improvement of protein quality and content in Sorghum bicolor (L.) Moench; annual report,1975/1976

3. AUTHOR(S)
(101) Purdue Univ. Dept. of Agronomy

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1976	90p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS
Purdue

8. SUPPLEMENTARY NOTES (Sponsoring Organization, Publishers, Availability)
(Research summary)

9. ABSTRACT

The protein quality of grain sorghum is limited by its low lysine content which reflects the high prolamine content of the endosperm. The tannin compounds present in the grain of some cultivars substantially reduce protein availability, which indirectly has a major negative effect on the nutritional quality of grain sorghum. During this year, significant progress has been made toward the understanding and alleviation of both of these limiting factors. Two high lysine sorghum lines from the tropical highlands of Ethiopia were identified by research conducted on this project. Inheritance studies demonstrated that the increased lysine concentration of each line is controlled by a single recessive gene. The biological value of the high lysine sorghum is double or triple that of average sorghum grain. A second high lysine gene mutation (P721) has been produced by chemical mutagen treatments of a photoperiod insensitive, broadly adapted, disease resistant sorghum variety. These and related discoveries provide the tools needed potentially to upgrade the nutritional status of sorghum grain.

10. CONTROL NUMBER
PN-AAC-774

11. PRICE OF DOCUMENT

12. DESCRIPTORS
Proteins
Sorghum

13. PROJECT NUMBER

14. CONTRACT NUMBER
AID/ta-C-1212 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

AID/ta-C-1212 Reg.
PN-AAC-774

Annual Report on Inheritance and Improvement of Protein Quality and Content in Sorghum

APRIL 1, 1975 – MARCH 31, 1976
PURDUE UNIVERSITY SORGHUM PROJECT
REPORT NO. 12
CONTRACT AID/ta-C-1212
UNITED STATES AGENCY FOR
INTERNATIONAL DEVELOPMENT
DEPARTMENT OF STATE
WASHINGTON, D.C.

ANNUAL REPORT

ON

"INHERITANCE AND IMPROVEMENT OF PROTEIN QUALITY AND CONTENT IN *SORGHUM BICOLOR* (L.) MOENCH"

April 1, 1975 - March 31, 1976

REPORT NO. 12

prepared by:

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Contract AID/ta-C-1212

Purdue University

Submitted to:

United States Agency for International Development
Department of State
Washington, D. C.

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REPORT SUMMARY

A. 1. Project Title and Contract Number: "Inheritance and Improvement of Protein Quality and Content in Sorghum bicolor (L.) Moench." No. PRF-AID/ta-1212.

2. Principal Investigator: John D. Axtell, Department of Agronomy. Contractor and Mailing Address: Purdue Research Foundation, Purdue University, W. Lafayette, Indiana 47907.

3. Contract Period : April 1, 1975 to March 31, 1978

4. Period covered by report: April 1, 1975 to March 31, 1976.

5. Total AID funding of contract to date: $299,978

6. Total expenditures and obligations through previous contract year: -0-

7. Total expenditures and obligations for current contract year: $299,978.

8. Estimated expenditures for the next contract year: (4-1-76 to 3-31-77): $319,098.

B. Narrative Summary of Accomplishments and Utilization: Grain sorghum is a major source of carbohydrate and protein for many millions of people in the developing world. It is generally recognized that the nutritional quality of many sorghums now being consumed is relatively poor when compared to other major cereals. This project has identified some of the problems responsible for this poor nutritional quality through a closely coordinated interdisciplinary approach by animal nutritionists, biochemists, and plant geneticists. Protein quality is limited by the low lysine content of the sorghum grain, which reflects the high prolamine content of the endosperm. The tannin compounds present in the grain of some cultivars substantially reduce protein availability, which indirectly has a major negative effect on the nutritional quality of grain sorghum. During the past year significant progress has been made toward the understanding and alleviation of both of these limiting factors.

Two high lysine sorghum lines from the tropical highlands of Ethiopia have been identified by research conducted on this project. Inheritance studies demonstrate that the increased lysine concentration of each line is controlled by a single recessive gene. The biological value of the high lysine sorghum is double or triple that of average sorghum grain. Recently a second high lysine gene mutation (P721) has been produced by chemical mutagen treatments of a photoperiod insensitive, broadly adapted, disease resistant sorghum variety. These and related discoveries provide the tools needed to potentially upgrade the nutritional status of millions of sorghum-consuming people in developing countries.

Three sets of entries in an International Protein Yield and Quality Trial nursery have been established at approximately 21 locations throughout the world to determine the adaptability of improved germplasm and to monitor progress made in the improvement of nutritional quality. Research information has been distributed through seminars, journals, publications, and annual reports. Graduate student and post-doctorate training programs continue to supply needed trained scientists for sorghum improvement programs in temperate and tropical areas of the world.

A. GENERAL BACKGROUND

Sorghum is the fourth most important cereal crop grown for human consumption in the world, being surpassed only by rice, wheat, and corn in worldwide importance. In Africa and much of Asia it is the principal source of energy for many millions of people and provides a major source of protein in the human diet. Though it is considered relatively high in carbohydrates and a fair source of total protein compared to most cereal crops, the protein quality is the poorest of the major cereals with regard to amino acid balance and total digestibility.

The purpose of this project is to develop and release for utilization in the LDCs superior varieties or lines of sorghum with genetically controlled higher protein content, improved amino acid balance and total digestibility. Thousands of lines from the world sorghum collection and the various breeding programs are being evaluated for levels of total protein and for quality by chemical and biological means. The more promising strains are recombined in various ways to improve further the amount and quality of protein in breeding populations. The parameters of inheritance of the high nutritional quality factors are in the process of being determined. Lines which do not produce seed at Purdue are sent to Puerto Rico for multiplication. There these lines are screened for improved yield for the tropics and seed returned to Purdue for screening of nutritional characteristics. Selected germplasm and supporting cultural practice information are provided to established programs and interested cooperators to test, screen, and to assist in the exchange of breeding materials. Graduate degree and non-degree training programs at

Purdue are developed and regularly conducted to increase the research capacity of developing nations with the desire to improve the nutritional quality of their sorghum.

B. STATEMENT OF PROJECT OBJECTIVES AS STATED IN THE CONTRACT.

Objective 1 - Identify and evaluate sorghum lines or mutants with improved protein quality and quantity utilizing both chemical and biological methods.

Objective 2 - Identify the chemical nature and composition of the protein fractions of selected high lysine sorghum genotypes and correlate the grain fraction composition with nutritional quality.

Objective 3 - Ascertain the role of various chemical components of sorghum grain such as polyphenols (tannins), protein quality, and carbohydrate availability to monogastric animal nutrition.

Objective 4 - Determine the mechanism of inheritance of high nutritional quality and develop breeding methods and materials including populations with improved biological quality for potential utilization in developing countries.

C. CONTINUED RELEVANCE OF OBJECTIVES.

During the past four years we have identified two distinctly different genes which improve overall protein quality and significantly enhance the potential biological value of the sorghum grain for human consumption.

The hl gene from Ethiopia represents a naturally occurring high lysine gene from sorghum entries in the world collection. Mutant P721 is a chemically induced high lysine mutant discovered during this past year.

The major challenge now is to develop high lysine germplasm adapted to the LDC's. A greater proportion of our total effort will, therefore, be expended in variety development utilization work.

D. ACCOMPLISHMENTS TO DATE

The major research achievement during the contract period has been the identification of a new high lysine mutant gene by D. P. Mohan which has better agronomic characteristics than the original hl gene from Ethiopia. This new mutant gene has been designated P721 and a description of its origin, inheritance, chemical composition, and biological value is presented in the following sections:

- Initial Survey of the Induced Mutants for Opaque Endosperm Mutants (p.4)
- Inheritance of High Lysine P721 Opaque and Its Amino Acid Composition (p.17)
- Biological Value of High Lysine P721 Opaque Mutant (p.31)
- Fractionation of Soluble Proteins of High Lysine Opaque P721 (p.37)
- Rat Feeding Experiment Comparing hl Sorghum IS 11758 with P721 Opaque (p.41)
- Comparison of Nutritional Value of High Lysine Grain Sorghum P721 Opaque and IS 11758 with Chicks (p.45)

Studies by W. R. Featherston, J. C. Rogler, and R. G. Elkin on the interference of tannins on sorghum grain utilization by the chick are summarized on pages 52-54.

Initial Survey of the Induced Mutants for Opaque Endosperm Mutants

It was hypothesized that higher lysine content in sorghum might be associated with an opaque endosperm phenotype similar to opaque-2 maize, and that these mutants could be induced by chemical mutagenesis. The frequency of induced high lysine barley mutants has been reported to be 1 per 750 lines screened, but only one mutant was reported out of 15,000 lines with a substantial increase (40%) in lysine content (Doll, 1973).

Mutation rates differ with regard to organism and genetic background. The spontaneous mutation rate at the opaque-2 locus was about one per 300,000 female gametes (Lambert and Alexander, 1968). Balint et al. (1970) found an opaque mutation rate of 1:6225 and 1:11846 in two strains. It was found that some mutants exhibited a change in amino acid pattern similar to opaque-2 mutation, but also a large number of opaque mutants were not associated with a change in amino acid composition.

Parental Germplasm

The parent lines 954114 and 954104 used in this study for the mutagen treatments are photoperiod insensitive, 3-dwarf with relatively

broad agronomic adaptability. The parent lines also had colorless pericarp and a translucent endosperm so that progeny from the mutagen treatments could be screened over a light box for identification of opaque mutant kernels. These lines were from partially converted Texas material bearing numbers TX4225-3, SC0170, PI276837.

The initial bulk material released in 1969 from which these parent lines have been derived was described in 1969 by Texas Agricultural Experiment Station and USDA as follows: "This bulk has good resistance to head smut, downy mildew, and anthracnose. It has erect leaves and stems that have excellent resistance to breakage after maturity. The white, somewhat pearly looking grain has good resistance to weathering. The plants are desirable agronomically and appear to have good yield potential."

Mutagen Treatments

Selfed seed* was treated with diethyl sulfate (DES) by soaking it in a solution for three hours. The treated seed (M_1 seed) was grown in Lafayette during 1972. Each M_1 head bearing M_2 seeds was bagged prior to anthesis to insure self-fertilization. Of the parent line 954114, 1407 fertile heads were recovered from the 1 ml DES/1000 ml of distilled water treatment and 387 fertile heads from the 2 ml DES/1000 ml of distilled water treatment. Of the parent line number 954104, 992 fertile heads were recovered from the treatment with 1 ml DES/1000 ml of distilled water and 214 heads from the 2 ml DES/1000 ml of water treatment. Each of these fertile heads bearing M_2 seeds were planted in single row plots so that a total of 3000 rows were grown in Puerto Rico during the winter of 1972-73. Each M_2 head bearing M_3 seeds was

* 300 g seeds were used in 4000 ml of water/treatment.

selfed and approximately 23,000 of these heads were harvested in the spring of 1973 in Puerto Rico and shipped to Lafayette for screening.

Each head was threshed individually, cleaned, and packeted in a coin envelope. A random sample of seeds was taken from each of the coin envelopes and examined over a light box for opaque mutant kernels. Approximately 14,000 heads were screened before the May 1973 planting at Lafayette and another 9000 heads were screened before the 1973-74 Puerto Rico winter planting.

Seed from each segregating head was separated into vitreous and opaque classes and given a putative mutant number (P number). Mostly the ratio of opaque to vitreous seed was 1:3, sometimes it was 1:1 and sometimes 1:15; other times no simple Mendelian ratios could be observed. Heads which were homozygous for opaque seeds needed no separation. Single analysis* of defatted whole kernel samples of opaque and vitreous class, or of the homozygous opaque class was done for nitrogen content by the micro-Kjeldahl procedure and converted to percent protein by multiplying nitrogen values by 6.25. Lysine was determined by ion-exchange chromatography. Protein is expressed as percent of dry sample and lysine as percent of protein as well as percent of dry sample.

Results and Discussion

Four hundred and seventy putative opaque mutants were identified over the light box on the basis of their opaque phenotype. There is

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* Shuman Chemical Laboratory, Inc., Battle Ground, Indiana

great variation in the protein and lysine content of the putative opaque mutants (Appendix Table 1). The protein content ranges from 7.6% in P672 opaque to 22.1% in P3 opaque. The range of lysine content (as percent of protein) is from 1.37 in P799 opaque to 4.93% in P750 opaque.

Table 1 illustrates the distribution of putative opaque mutants in terms of their relative increase in lysine content when compared to normal vitreous control sib seeds. As a control for the homozygous opaque seeds an average of all (445) normal sibs was taken which was equal to 14.4% protein and 1.9% lysine (as percent of protein). Lysine expressed as percent of sample was equal to .274. The comparison for increase in lysine content for homozygous opaques was with this number.

Most of the putative opaque mutants (Table 1) had only a slight increase in lysine content. The variation in protein and lysine content among opaque mutants parallels the situation in corn where many genes express opaque endosperm phenotypes but only opaque-2, floury-2 and opaque-7 have a pronounced effect on increased lysine content.

This study is confined to those opaque mutants which have had an increase of lysine concentration of more than 50%. Twenty-eight of these mutants (Table 2) were planted in the greenhouse or field at Lafayette or in Puerto Rico. Seven of these mutants were not planted because the seed size was too small and the high protein levels were probably due to this reason. From four of these mutants no opaques could be recovered in their progeny. With the exception of these, the high lysine mutants in Table 2 can be placed into two classes, Class I opaque mutants were those which produced phenotypically normal plants

Table 1. Distribution of putative opaque mutants in terms of their relative increase or decrease in lysine content when compared to normal vitreous control sib seeds.

Percent Increase in Lysine (% of sample)	Number of putative opaque mutants
Decrease	32
0-9	114
10-19	118
20-29	88
30-39	57
40-50	26
>50	35
Total	470

Table 2. Thirty-five putative opaque mutants with increase in lysine concentration of more than 50 percent.

Mutant No.	Puerto Rico origin	Percent protein	Lysine % protein	% Lysine % sample	Lysine increase	Germination	Phenotype: Kernel endosperm	Phenotype: Plant	Phenotype: fertile/sterile	Class	Trt.	Parent line	opaque:vit. ratio	Comments
P30 normal*	47058	11.4 12.6	3.50 1.78	.399 .224	178	poor	opaque vitreous	normal	sterile	II	2 ml DES	954114	164/426	
P31 normal	47058	10.8 11.2	3.48 1.95	.376 .218	172	none	opaque vitreous	—	—	II	2 ml DES	954114	251/722	
P43 normal	50038	16.7	2.51	.418	152	none	homozygous opaque	—	—	II	2 ml DES	954114	—	
P45 normal	50065	13.9 12.8	2.96 1.84	.410 .235	174	normal	opaque vitreous	grassy	—	II	1 ml DES	954114	106/463	
P46 normal	50065	14.5 14.6	3.26 1.84	.473 .269	175	normal	opaque vitreous	grassy	—	II	1 ml DES	954114	69/318	
P81 normal	53031	15.9 17.0	2.51 1.41	.399 .240	166	poor	opaque vitreous	normal	sterile	II	2 ml DES	954114	95/285	
P139 normal	40079	13.7 12.5	2.75 1.97	.370 .245	151	poor	opaque vitreous	normal	fertile	—	1 ml DES	954114	49/356	No opaques recovered in the progeny
P171 normal	37082	20.1 19.3	2.32 1.57	.465	153	poor	opaque vitreous	normal	sterile	II	1 ml DES	954114	27/87	
P597 normal	18103	16.3 13.4	2.58 1.79	.620 .240	175	normal	opaque vitreous	normal	fertile	—	1 ml DES	954104	67/576	No opaques recovered in the progeny
P629 normal	15073	16.8 16.0	3.45 1.75	.580 .280	207	none	opaque vitreous	—	fertile	II	1 ml DES	954104	90/606	
P720 normal	08006-1	18.5 18.1	3.16 1.74	.585 .315	185	normal	opaque vitreous	normal	fertile	I	1 ml DES	954114	Information not available	
P721 normal	08006-2	15.7 14.5	2.99 1.90	.470 .275	170	normal	opaque vitreous	normal	fertile	I	1 ml DES	954114	"	
P726 normal	08093-2	15.9 10.9	2.63 2.40	.417 .262	159	normal	opaque vitreous	normal	fertile	—	1 ml DES	954104	"	No opaques recovered in the progeny
P728 normal	09009-2	20.0 19.3	2.49 1.57	.498 .303	164	—	opaque vitreous	Not planted			1 ml DES	909114	"	
P750 normal	13002	17.2 16.5	4.93 2.29	.848 .378	224	none	opaque vitreous	—	—	II	1 ml DES	909114	"	
P829 normal	27097-1	18.0 16.2	2.43 1.78	.436 .287	152	poor	opaque vitreous	normal	fertile	—	1 ml DES	954104	"	No opaques recovered in the progeny

Table 2. (Continued).

Mutant No.	Puerto Rico origin	Percent protein	Lysine % protein	Lysine % sample	Lysine increase	Germination	Phenotype: Kernel endosperm	Phenotype: Plant	Phenotype: fertile/sterile	Class	Trt.	Parent line	opaque:vit. ratio	Comments
P831 normal	28003	20.8 19.7	2.46 1.49	.512 .293	174	—	opaque vitreous	Not planted			1 ml DES	954114	Information not available	
P843 normal	30076	17.0 15.9	2.65 1.66	.450 .264	170	—	opaque vitreous	Not planted			1 ml DES	954114	"	
P851 normal	32094	12.0 11.7	3.29 2.00	.395 .234	168	—	opaque vitreous	Off type plants		—	1 ml DES	954114	"	
P863 normal	36004	14.7 12.5	4.58 1.96	.672 .245	274	none	opaque vitreous	—	—	II	1 ml DES	954114	"	
P864 normal	36077	16.1 16.2	2.62 1.66	.422 .269	156	none	opaque vitreous	—	—	II	1 ml DES	954114	"	
P865 normal	36096	17.5 16.8	2.74 1.68	.479 .281	170	poor	opaque vitreous	grassy	—	II	1 ml DES	954114	"	
P866 normal	36103	12.3 11.4	2.66 1.76	.326 .201	162	poor	opaque vitreous	Planting mistake		—	2 ml DES	954104	"	
P867 normal	36105	11.7 11.3	3.88 2.75	.454 .253	179	none	opaque vitreous	—	—	II	2 ml DES	954104	"	
P868 normal	37083	18.0 17.5	2.33 1.50	.418 .262	159	none	opaque vitreous	—	—	II	1 ml DES	954114	"	
P884 normal	41096	14.9 15.4	2.92 1.62	.435 .248	175	poor	opaque vitreous	shorter	fertile	II	1 ml DES	954114	"	
P899 normal	44008	19.0 18.1	2.64 1.53	.502 .277	181	normal	opaque vitreous	seg. ht.	fertile	II	1 ml DES	954114	"	
P908 normal	45090	14.1 11.0	4.82 2.06	.680 .227	299	none	opaque vitreous	—	—	II	1 ml DES	954114	"	
P926 normal	46070	20.9 20.0	2.36 1.51	.492 .302	163	—	opaque vitreous	Not planted		—	1 ml DES	954114	"	
P928 normal	47022	16.0 14.5	2.38 1.56	.381 .225	169	—	opaque vitreous	Not planted		—	2 ml DES	954114	"	
P938 normal	47049-1	17.2 17.2	2.65 1.66	.456 .285	160	none	opaque vitreous	—	—	II	2 ml DES	954114	"	
P946 normal	47049-2	15.2 12.8	2.52 1.44	.382 .183	208	—	opaque vitreous	Not planted		—	1 ml DES	954114	"	

Table 2. (Continued).

Mutant No.	Puerto Rico origin	Percent protein	Lysine % protein	Lysine % sample	Lysine increase	Germination	Phenotype: Kernel endosperm	Phenotype: Plant	Phenotype: fertile/ sterile	Class	Trt.	Parent line	opaque:vit. ratio	Comments
P952 normal	48040	18.0 17.8	2.38 1.56	.427 .278	153	—	opaque vitreous	Not planted		—	2 ml DES	954114	Information not available	
P994 normal	50106	14.9 14.1	3.12 1.77	.465 .249	187	poor	opaque vitreous	shorter	—	II	2 ml DES	954104	"	
P1021 normal	52060	13.8 13.7	2.88 1.90	.396 .259	152	none	opaque vitreous	—	—	II	2 ml DES	954114	"	

* Normal refers to segregating vitreous seed derived from the same head.

when grown in Lafayette or Puerto Rico in 1973. Class II mutants on the other hand produced abnormal plant growth ranging from chlorophyll deficiency through dwarf or stunted plants. Plants from these opaque kernels seem to have lost apical dominance and produce numerous short tillers, but no flowers or seeds. Plants grown from normal sib seeds from the same head produced normal plants, which suggests that the opaque mutant itself is responsible for the abnormal sporophytic effects. That is, the grassy phenotype is probably a pleiotropic effect of the mutation producing opaque seed, but close linkage cannot be excluded at this time. There are many examples of Class II mutants (Table 2) where there was no germination, whereas normal plants were obtained from vitreous sib seed from the same head.

The class I high lysine opaque mutants produced plants with normal phenotypes and since these are most useful agronomically they were studied further. M_4 seeds of P720 and P721 were screened for homozygous opaque seeds over the light box. Fourteen heads of P721 and one head of P720 were found to be homozygous opaque. Seeds of these 14 homozygous opaque heads from the 1973-74 Puerto Rico planting were analyzed for protein and lysine content. Seed from one homozygous opaque head of P720 was also analyzed (Table 3). The average whole grain lysine content of P721 opaque was 2.8 (expressed as percent of protein) at 17.5% protein relative to the normal sib average of 1.7% lysine (expressed as percent of protein) at 15.4% protein. The average whole grain lysine content of P720 opaque was 2.9% (expressed as percent of protein)

Table 3. Protein and lysine content of defatted whole kernels of P721 and P720 from Puerto Rico 1973-74 planting.

Entry No.	Protein %	Lysine (g/100g P)	Lysine (% of sample)
		P721 OPAQUE	
444 427	11.9	2.42	.288
439	18.2	3.01	.548
447	17.9	2.95	.528
458	14.4	2.70	.389
470	20.1	3.09	.621
471	13.3	2.84	.378
476	22.2	3.04	.675
481	18.9	2.90	.548
485	19.2	2.92	.561
496	17.2	2.75	.473
507	20.5	2.78	.570
515	16.0	2.68	.429
525	15.3	3.14	.479
532	20.4	2.71	.553
Mean	17.5	2.84	.503
		P721 NORMAL	
537	16.9	1.24	.209
539	16.0	1.48	.237
545	14.7	1.63	.240
557	12.3	1.69	.208
563	13.5	1.92	.259
575	16.9	1.50	.253
582	16.0	1.57	.251
589	13.3	1.91	.253
597	13.9	1.85	.256
607	15.9	1.93	.307
611	16.5	1.73	.285
616	18.2	1.75	.318
618	15.8	1.39	.220
Mean	15.4	1.70	.253
		P720 OPAQUE	
412	16.5	2.90	.479
		P720 NORMAL	
757	15.8	1.74	.275
768	14.4	1.65	.238
Mean	15.1	1.7	.257

at 16.5% protein compared to the normal sib average of 1.7% (expressed as percent of protein) lysine at 15.1% protein.

In 1974, P721 was increased at Lafayette and protein and lysine content of 14 sib families was analyzed (Table 4). The average lysine content was 3.09% of protein at an average protein content of 13.9%. The lysine concentration expressed as percent of sample was .432. Eight families from normal sibs were also increased in 1974 at Lafayette and analyzed for protein and lysine content (Table 4). The average lysine content was 2.09% of protein at an average of 12.09% protein which is about the average for lines in world sorghum collection (Srinivasan et al. 1972). The increase in lysine content over the normal sib is 60%.

As the seeds of P720 and P721 came originally from the same head row (see Table 2) it is presumed that they carry the same mutation for high lysine gene though the test for allelism has not been made.

On the basis of these preliminary observations it was hypothesized that P721 opaque is a gene mutation which alters the normal amino acid pattern in the endosperm. Similar gene mutations have been reported in corn, barley and sorghum (Mertz et al. 1964; Munck et al. 1970; Singh and Axtell, 1973).

These observations provided the basis for further chemical, biological evaluation, inheritance, and, endosperm morphological studies.

Summary and Conclusions

After screening 23,000 M_2 sorghum heads over a light box which were progeny from DES mutagen treatments, 475 possible opaque mutants were identified. One mutation, P721 opaque, was normal phenotypically

Table 4. Protein and lysine content of defatted whole kernels of P721 and P720 from Lafayette 1974 planting.

Entry No.	Protein %	Lysine (g/100g P)	Lysine (% of sample)
		P721 OPAQUE	
444 427	12.8	3.11	.398
439	14.0	3.10	.434
447	14.0	3.01	.421
458-A	14.4	3.18	.458
458-B	14.4	3.08	.443
470	14.1	3.22	.454
471	13.7	3.11	.426
476	13.3	2.99	.398
481	13.9	3.12	.434
485	14.2	3.41	.484
496	13.5	2.96	.400
507	14.3	3.16	.452
515	13.4	3.00	.402
525	14.6	3.00	.438
532	14.7	3.16	.464
Mean	13.9	3.09	.432
		P721 NORMAL	
444 539	14.3	1.93	.276
545	13.3	1.93	.257
551	12.8	2.07	.265
574	12.1	2.34	.283
575	13.5	1.96	.265
582	12.8	2.21	.283
597	12.6	2.16	.273
607	12.2	2.12	.259
Mean	12.9	2.09	.270

and had a 60% increase in lysine content relative to the normal sib.

This preliminary observation suggested that P721 opaque may be the result of a gene mutation associated with better nutritional quality similar to the hl gene from Ethiopia. This was the basis for further studies related to chemical, biological evaluation, genetic inheritance and endosperm structure of P721 opaque.

Inheritance of High Lysine P721 Opaque and Its Amino Acid Composition

Inheritance Study

Plants grown from P721 opaque seeds and from the normal sib were grown during the winter of 1973-74 at Puerto Rico. Crosses were made with P721 opaque pollen on cytoplasmic male sterile combine kafir 60, male sterile KS 24 and genetic male sterile plants from Purdue population PP3R. The F_1 progenies were grown at Lafayette and 10 heads were selfed to obtain F_2 seeds for chemical analysis. Selfing was also done to check for restorer or non-restorer reaction in the case of cytoplasmic male sterile crosses with P721.

A dosage series was prepared by selfing the homozygous parents Martin and P721 opaque and making the reciprocal crosses between these lines during the summer of 1974 at Lafayette. A genetic ms derivative of Martin was used to facilitate crossing. Crosses on P721 opaque were made by hand emasculation. The F_1 seeds were then analyzed for protein and lysine content.

A preliminary test for allelism was performed by making crosses between P721 opaque and lines homozygous for the hl gene from Ethiopia. These F_1 seeds were also analyzed for lysine and protein content.

Heads segregating for opaque and vitreous classes of seeds of P721 were available from 1973-74 Puerto Rico harvest and 1974 Lafayette harvest to study the inheritance of the floury endosperm mutant P721. Chi-square tests were performed to test goodness of fit to a theoretical ratio.

Chemical Evaluation

The crude protein content of whole kernel and endosperm samples of P721 was determined by micro-kjeldahl (A.O.A.C., 1960) nitrogen times the factor 6.25. All samples were defatted, hydrolyzed and analyzed for amino acid content using an automatic Beckman Model-120C ion-exchange resin chromatography amino acid analyzer except for methionine, cystine and tryptophan which were analyzed separately.

Methionine and cystine were determined by method described by Moore (1963); tryptophan by the method of Slump and Schrender (1969).*

Percent oil in whole grain samples of P721 was determined by nuclear magnetic resonance spectroscopy (NMR) at the University of Illinois, Urbana, Illinois.

Catechin equivalents were determined as percent of sample both by using the vanillin-HCl procedure described by Burns (1971) by the use of methanol extract, and, the modified vanillin-HCl procedure (Maxson and Rooney, 1972) by the use of acidified methanol extract.

Plant height and number of days to 50% flowering were recorded. One hundred seed weight was calculated by weighing 100 random seeds. Germ and endosperm tissue of 100 whole kernels were separated by hand

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* Shuman Chemical Laboratory, Inc., Battle Ground, Indiana.

dissection without removal of pericarp for estimating their proportions by weight and for chemical analysis.

Results and Discussion

Inheritance of High Lysine Character

Phenotypic Segregation

Preliminary results of the inheritance study described here suggests that the high lysine character is probably simply inherited. Table 5 shows the distribution of vitreous and opaque kernels from three F_2 panicles grown from plants heterozygous for opaque mutant P721. Chi-square analysis indicates a reasonable fit to a 1:1 ratio in some cases but not in others. A semi-dominant mode of inheritance is suggested for P721 opaque as is true for floury-2 in corn where each successive dose of floury-2 in the endosperm adds an additional increment of lysine (Bates, 1966).

F_2 segregation data from three additional crosses is presented in Table 6. The female parents used as normal was CK60A, KS24A and PP3R $\underline{ms}_3$. The pollen parent was heterozygous for the opaque character. The F_1 seed obtained from the crosses was planted and F_2 seed from segregating heads was classified for opaque and vitreous endosperm by sectioning individual kernels. F_2 data from the cross with CK60A gave a satisfactory fit to 1:1 ratio. The other two crosses have more opaque than vitreous seeds. In fact, a ratio of 3 opaques to 1 normal will give a good fit in the second cross. For PP3R $\underline{ms}_3$, no simple ratio is obvious. All this variation could perhaps be explained by gene dosage effects in the triploid endosperm and modifiers from different genetic backgrounds.

Table 5. Classification of kernels from three branches of self-pollinated panicles segregating for opaque mutant P721.

Pedigree	No. of Kernels			X^{2*}	Prob.
	Vitreous	Opaque	Total		
443 176-1	144	158	302	0.648	.25 - .50
443 176-2	114	116	230	0.016	.90 - .75
443 119-1	137	157	294	1.360	.25 - .10

* Based on 1:1 expected ratio.

Table 6. F_2 segregation and chi-square values for crosses involving P721 opaque.

Cross	Total number of panicles threshed	Total number of heads	No. of Kernels Opaque	Vitreous	Total	X^{2*}
CK60A x P721 (+/o)	6	6	272	252	524	0.76
KS24A x P721 (+/o)	5	2	307	99	406	106.56
PP3R$\underline{ms}_3$ x P721 (+/o)	50	11	2431	1795	4226	95.71

* Except for first cross values were significant ($P<0.05$) indicating a nonsatisfactory fit to the 1:1 ratio.

Preliminary results of dosage series are shown in Table 7. There seems to be a dosage effect of the high lysine opaque gene in P721. Two doses of the opaque gene (+/o/o) increase the lysine content whereas one dose (+/+/o) has no effect. Results were preliminary since few seeds were available for analysis from hand emasculated crosses and the germ was not separated from the endosperm. There was also an untimely frost during the maturation of these seeds.

A preliminary test for allelism was performed with the high lysine mutants IS11758 and P721 opaque. All F_1 seeds appeared to be vitreous indicating the nonallelic nature of the two genes though there were a few opaque seeds perhaps because of the early frost. Table 8 shows the lysine and protein content of cross between P721 opaque and lines homozygous for hl gene and also a line considered as a normal check from the random mating Purdue Population PP20. There is no indication of an increase in lysine content which would be expected if these two mutant genes were allelic.

Amino Acid Composition of Defatted P721 Opaque and Normal Sib Kernels

Similar to the opaque-2 mutant of corn (Nelson, 1966) the effect of high lysine gene P721 opaque does not appear to affect the proteins produced in the embryo. Table 9 presents protein and lysine contents of defatted embryo of P721 opaque and normal sib and also the proportion of embryo and endosperm in these two classes of kernels. The lysine content in both classes of embryo was similar, though there was some increase in percent protein in the mutant class.

Table 7. Dosage effect of P721 opaque gene (Lysine g/100g whole kernel protein).

Martin ms_1 used as normal parent	Martin ms_1	Martin ms_1 x P721 opaque	P721 opaque x Martin ms_1	P721 opaque
	+ + +	+ + o	+ o o	o o o
Lysine	2.37	2.32	2.90	3.24

Martin ms_3 used as normal parent	Martin ms_3	Martin ms_3 x P721 opaque	P721 opaque x Martin ms_3	P721 opaque
Lysine	2.22	2.24	3.26	3.23

* o is abbreviation for the P721 opaque gene.

Table 8. Test for allelism performed for two high lysine mutants IS11758 and P721 opaque.

Cross	Percent protein	Lysine (% of protein)
Female Male		
hl hl x P721 opaque	17.5	2.05
PP20 x P721 opaque	19.3	1.91

Table 9 . Protein and lysine content of defatted embryo and endosperm and the proportion of embryo and endosperm.

Kernel class	Part of kernel	Protein percent	Lysine (% of protein)	Proportion (% of seed)
P721 opaque	embryo	27.7	5.37	11.4
Normal sib	embryo	23.8	5.36	10.4
P721 opaque	endosperm	12.5	2.06	88.6*
Normal sib	endosperm	11.7	1.25	89.6*

* Pericarp was not removed from endosperm.

The embryo comprised 11.4% of the P721 opaque kernel and 10.4% of the normal sib kernel. The difference in lysine content of both kernel classes were more pronounced in the endosperm tissue. The lysine content (as percent of protein) of the endosperm of the normal sib was 1.25 compared to 2.06 for the endosperm of P721 opaque kernels. This is an increase of 64% in the lysine content of the latter.

The amino acid composition of the defatted whole kernel and endosperm classes of P721 opaque and normal seeds is shown in Table 10. There is an increase in lysine and arginine concentration, and a decrease in glutamic acid, alanine and leucine concentration in opaque seeds relative to normal vitreous sib seeds. There is also an increase in the percent protein of opaque kernels. The shift in amino acid pattern of P721 opaque endosperm tissue is comparable to that reported for opaque-2 maize endosperm (Nelson, 1969) and hl endosperm in sorghum (Singh and Axtell, 1973).

The desirable changes nutritionally are increases in lysine content and decreases in leucine content and thereby a reduced leucine to isoleucine ratio. The low leucine to isoleucine ratio may contribute to antipellagragenic properties as has been confirmed for opaque-2 corn, (Gopalan et al. 1969). These changes in amino acid content coupled with increase in protein content should make P721 opaque a better food and feedstuff for humans and animals than ordinary sorghum.

Chemical Composition and Agronomic Traits of P721 Opaque

A comparison of P721 opaque characteristic with normal sibs and also with IS11758 and normal sorghum is shown in Table 11.

Table 10. Amino acid (g/100g protein) and protein content of defatted kernels of P721 opaque and normal sib sorghum lines.

Amino acid	Whole Kernels P721 opaque[1]	Normal sib[1]	Avg.* sorghum	Avg.‡ sorghum	Endosperm P721 opaque[2]	Normal sib[2]
Lysine	3.24	2.38	2.14	2.00	2.16	1.20
Histidine	7.49	2.35	2.01	2.55	2.16	1.88
Arginine	5.29	4.07	3.59	3.60	3.44	2.65
Aspartic acid	7.85	7.31	7.83	8.20	7.28	5.73
Threonine	3.38	3.66	3.26	3.60	2.96	2.74
Serine	4.13	4.59	4.52	4.95	3.36	3.76
Glutamic acid	19.34	22.02	23.22	26.95	19.36	21.62
Proline	7.55†	8.69†	8.16	9.05	9.36	9.83
Glycine	3.60	3.41	3.07	2.95	2.64	2.31
Alanine	7.36	8.61	9.89	10.95	7.52	8.55
Cystine	1.93†	1.97†	1.50**	1.75	1.84	1.79
Valine	5.52	5.44	5.35	5.70	5.44	5.04
Methionine	2.03†	1.97†	1.80**	1.35	2.00	1.62
Isoleucine	4.10	4.21	4.08	4.60	4.24	4.19
Leucine	11.47	13.66	14.27	16.70	12.88	14.19
Tyrosine	4.41	4.70	4.50	5.30	4.80	4.62
Phenylalanine	5.12	6.13	5.19	6.00	5.90	5.52
Tryptophan	-	-	1.31***	-	-	-
% Protein	14.30	12.20	12.60	9.30	12.50	11.70
Leucine/ isoleucine	2.80	3.24	3.50	4.60	3.03	3.39

1 The amino acid values for whole kernels and endosperm are the averages of three determinations from separate hydrolyzates.
2 From single determination only.
* Weighted average of 522 lines (Srinivasan et al. 1972).
** Weighted average of 3 lines.
***Weighted average of 9 lines.
‡ Average of two lines (Jambunathan and Mertz, 1972).
† Average of two determinations from separate hydrolyzates.

Table 11. Comparison of high lysine line P721 opaque with various characters of IS11758, normal sib and normal sorghum.

Character	Puerto Rico		Lafayette		
	P721	Normal sib	P721	Normal sib	IS11758*
Protein (%)	17.5	15.4	13.9	12.9	17.20
Lysine (% of protein)	2.8	1.7	3.09	2.09	3.13
Lysine (% of sample)	0.49	.262	0.48	.270	0.54
Seed weight (g/100 seed)	--	--	1.81	2.28	2.45
Protein per seed (mg)	--	--	2.52	2.9	4.21
Lysine per seed (mg)	--	--	0.078	0.062	0.13
Percent germ	--	--	11.4	10.4	16.30
Oil (%)	--	--	4.45	3.62	6.61
Catechin equivalent					
methanol ext.	--	--	0.09	0.09	0.37
acidified methanol	--	--	0.21	0.17	--
Height (cm)	110.0	--	100.0	--	245 (P.R.)
Days to 50% flowering	72	--	85	--	photoperiod sensitive
Restorer or non-restorer	Restorer				nonrestorer
Yield estimate	No information				No information

* (Singh, 1973).

Besides the higher lysine content (47-64% higher than normal sib) of P721 opaque it is also higher in protein content than the normal sib by 7 to 13%. The higher percentage of lysine in protein of this line coupled with the high protein content of the grain elevates lysine content when expressed as percent of sample by 73% over the normal sib. The average test weight of P721 opaque is 25% less than the normal sib. The average protein content expressed on a per seed basis is therefore less by 15% when compared to its normal sib. However, the average lysine content per seed is 25% higher than the normal sib. The oil content is increased by 22% over the normal sib.

Tannin expressed as catechin equivalents in P721 by the method of methanol extraction and acidified methanol extraction have low values that fall below the level known to interfere with protein availability (Oswalt, 1973, 1975). The height of P721 is between 100-110 cms. The 50% flowering period is between 72-85 days. The P721 opaque line restores fertility to the cytoplasmic male sterile used, namely CK60A and KS24A.

A comparison of P721 opaque with IS11758 which is the best high lysine line known at this time shows that P721 opaque has (averaged over two locations) 9% less protein content, a 6% lower lysine content and 17% less lysine content expressed as percent of sample. The fact that P721 opaque is a photoperiod insensitive line and therefore has a wider adaptability is a distinct advantage. Another point is that its seed is not colored while IS11758 seed is orange. P721 opaque would be preferred over IS11758 wherever sorghum is eaten especially in India and Sudan.

Summary and Conclusions

A high lysine gene designated P721 has been found from chemical mutagenesis. It is inherited as a semi-dominant. This gene causes a major increase in lysine, and arginine concentration and a decrease in glutamic acid and leucine concentration, when compared to normal sib seed. There is also an increase in germ size compared to normal sorghum.

A preliminary allelism test indicates that this gene is different from the hl gene reported by Singh and Axtell (1973). The higher lysine content of P721 opaque should improve the nutritional value in lines or hybrids containing this mutant gene.

Biological Value of High Lysine P721 Opaque Mutant

Based on the improved amino acid composition of P721 opaque seed it needed to be known if it had a better biological value than normal sorghum. To test this hypothesis, a 28-day rat trial was planned to compare the feeding value of P721 opaque, the normal sib and a ANRC casein diet as control.

A rat feeding experiment* was done from seeds increased at Lafayette during 1974 to compare the biological value of high lysine P721 opaque, normal sib and ANRC casein.

Sixty 21-day old male albino weanling rats (Rattus rattus) belonging to the Spague-Dowley strain with nearly similar initial weight were used for the evaluation of 3 different diets. Rats were arranged in groups of 20 to make their average weight close to their overall mean weight and were kept one rat per cage in wire-mesh cages. Experimental diets were fed each rat for 28 days.

Individual rat weight gains and their feed consumption was measured after the 1st, 2nd, 3rd and 4th week of the experiment. The use of

* Experiment was conducted by Shuman Chemical Laboratory, Inc., Battle Ground, Indiana.

special feed cups minimized the feed spill to a negligible level. Rats were given unrestricted access to the diet and water was offered ad libitum. Water was changed on alternate days. Rat weight gain, feed consumption, feed efficiency ratio (FER, feed consumed per gram of body weight gain), and protein efficiency ratio (PER, weight gain per gram of protein consumed) data were calculated.

Preparation of Diets

Seed samples used for the preparation of diets were all from selfed seeds to guard against the changes in chemical composition that would be effected by foreign (non-opaque) pollen (Bates, 1966). Crude protein (N x 6.25) level of these sorghum lines ranged from 12.9 to 13.9%. Feeds were made isonitrogenous to 10% protein level by dilution with starch since differences between protein quality tends to disappear at higher levels of protein intake (McLaughlan and Campbell, 1969). Celluflour and corn oil were added to adjust to approximately equal levels of fibre and fat. Thus each 100 g of basal diet consisted of varying amounts of sorghum grain or casein and corn starch plus 4 g of mineral mixture* and 2 g of vitamin mix.** Each diet was analyzed for protein and lysine.

Experimental Design

The experiment was considered as a completely random design. Weight gain data was statistically analyzed as described by Steel and Torrie (1960).

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* Hawk-Oser Salt mixture number three, Nutritional Biochemicals, Inc., Cleveland, Ohio.

** Vitamin supplement, General Biochemicals, Chagrin Falls, Ohio.

Results and Discussion

All the diets were prepared at isonitrogenous level of about 10% protein. However, protein analysis data (Table 12) showed some difference in protein levels of various diets.

Data on the chemical composition of whole grain samples and composition of the diets as well as rat weight gain, feed efficiency ratio (FER) and protein efficiency ratio (PER), are given in Table 12. The average initial weight of weanling rats was 43.98 g. There was no significant difference in average initial weight between groups of rats placed on different diets. Weight gain on the P721 opaque diet was nearly three times higher than the normal sib weight gain. The PER values for P721 opaque rations was higher than the PER for normal sib rations but was lower than that for the casein diet. Weight gains at 7-day intervals through the 28-day feeding trial are shown in Figure 1.

This experiment proves that the high lysine sorghum P721 opaque is superior in biological value to normal sorghum. The normal sib is very close in chemical composition to the average of world sorghum. Therefore, P721 opaque sorghum is superior in biological value to normal grain sorghum. However, the comparison of the biological value of P721 opaque with high lysine line IS11758 is dealt in a separate section later on.

It may be questioned whether results of improved biological value of P721 opaque as noticed with rats can be applied directly to human diets. Animal assays provide only a relative index of the qualitative nature of the protein in food (Yang and Mickelsen, 1974). On the other hand, Hegsted (1957) has shown that there is a remarkably good agreement

Table 12. Biological values of isonitrogenous (10% protein) diets prepared with high lysine P721 opaque and normal sorghum lines in a 28-day rat feeding experiment.

Source	Composition of grain	Composition of feed					
			Lysine				
	Protein percent	Protein percent	g/100 g protein	percent of sample	Weight gain (g) 28-days	FER	PER
P721 opaque	12.4	10.25	3.01	.309	32.69	5.7	1.8
Normal sib	11.4	10.0	2.15	.215	10.95	10.6	0.9
Casein	91.0	10.95	8.26	.905	77.4	3.9	2.8
C.V., %					21.4		
S.E. of treatment mean					2.0		
LSD 5% (between individual line mean)					5.8		
LSD 1% (between individual line mean)					7.8		

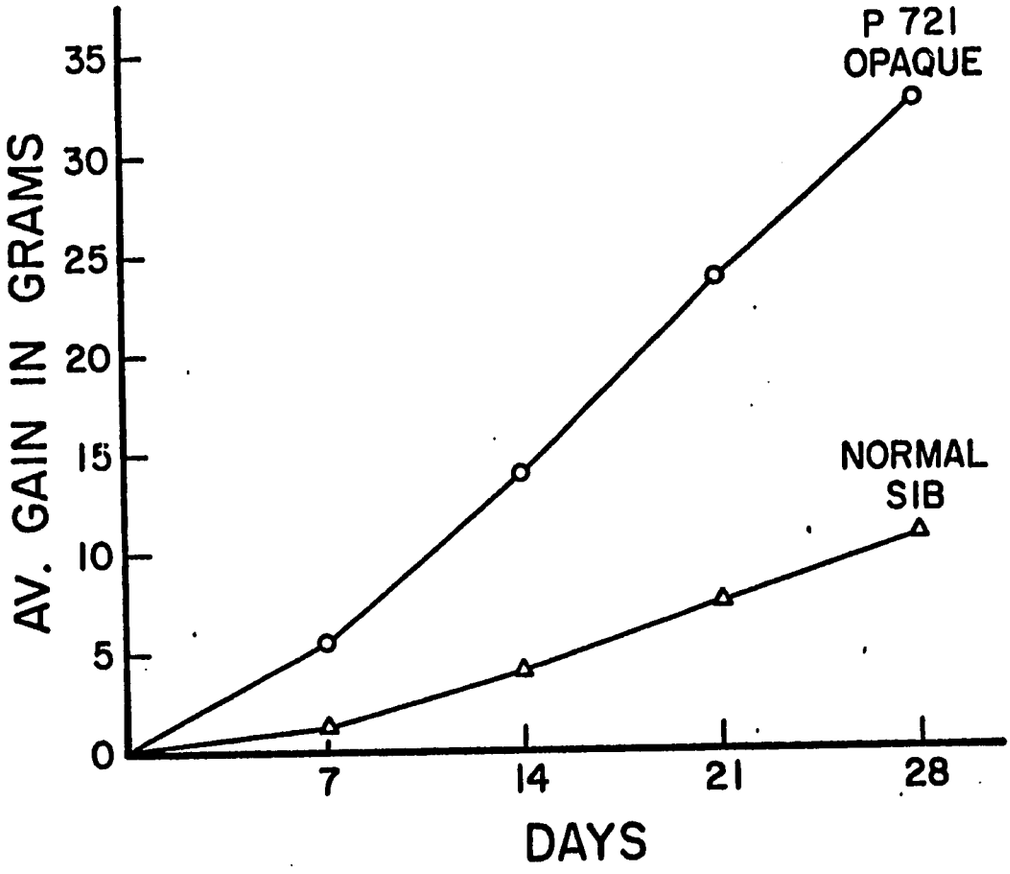

Figure 1. Average weight gain of rats fed on P721 opaque and normal sib rations at 10% protein level.

between observed protein requirements for animals and man. Howe et al. (1965) state as the rat is a rapidly growing animal, its nutritional requirements may be more stringent than those of man. Therefore, a protein which performs well for weanling rats will almost certainly prove satisfactory for infants (Howe et al. 1965). The better nutritive value of opaque-2 corn protein for humans has been shown by Bressani, 1966; Clark et al. 1967; Harpstead and Sarria, 1968), for pigs (Pickett, 1966; Cromwell et al. 1967). Therefore, the better biological value of high lysine opaque P721 as observed with rats should also be superior to normal sorghum protein in diets of man and domestic animals.

Summary and Conclusions

High lysine P721 opaque mutant has many desirable changes in its amino acid composition. When diets prepared from P721 opaque were fed to weanling rats at isonitrogenous (10% protein) levels the average rat weight gain was three times more than the normal sib. Protein efficiency ratio (PER) for P721 opaque was 1.8 as compared to 0.9 for the normal sib. Protein quality of the opaque mutant is therefore twice as good as the normal sib. Feed consumption of rats per gram of weight gain (FER) was 5.7 as compared to 10.6 of the normal sib.

Based on more stringent nutritional requirements of weanling rats than those of humans, any protein which performs well in weanling rats would do well with humans. It is expected that high lysine sorghum should be superior to normal sorghum protein in the diets of man and domestic animals.

Fractionation of Soluble Proteins of High Lysine Opaque P721

In opaque-2 corn the prolamine (zein) fraction is reduced and the glutelin, albumin and globulin fractions are increased resulting in an increase in lysine of the whole kernel (Jimenez, 1966, 1968; Mossé, 1966). The protein distribution patterns obtained with high lysine sorghum (hl) as reported by Jambunathan et al. 1975, are similar to opaque-2 maize.

The objective of this study was to study the protein fractionation data of high lysine sorghum mutant P721 opaque.

Two samples each of selfed seed of P721 opaque and normal grown at Purdue in 1974 were used.

The endosperm, germ and pericarp (bran) fractions enough for two chemical analyses were separated by using the procedure of Hubbard, Hall, and Earlie (1950). The separated fractions were air dried at room temperature to a constant weight and the percent distribution of each of the

* All experiments in this section were done by Biochemistry Department, Purdue University under the direction of Dr. E. T. Mertz and technical assistance of Ms. Lourdes Tanchoco.

fractions calculated. The endosperm portions were ground and defatted. Protein was fractionated according to the Landry-Moureaux procedure (Landry and Moureaux, 1970) as previously applied to sorghum (Jambunathan and Mertz, 1973).

Results and Discussion

Table 13 gives the percent of endosperm, germ and pericarp expressed as percent of whole kernel. It is seen that germ of P721 opaque is about 11% larger than normal sib and average sorghum. The relative increase in germ size must be adding to the improvement of nutritional quality of P721 opaque kernel.

Table 14 gives the percentage distribution of nitrogen in five fractions obtained from the endosperm. The percent of soluble nitrogen is increased in P721 opaque in the first and fifth fractions and decreased in the second and third fractions when compared to normal sib endosperm.

The first fraction represents the saline soluble proteins, albumins, globulins and free amino acids. It has the highest amount of lysine in comparison to other protein fractions, and any increase in its percentage distribution would increase the percent of lysine in the endosperm. Fraction II represents the alcohol-soluble proteins (kafirins) solubilized by isopropanol. Fraction III represents the proteins soluble in isopropyl alcohol containing a reducing agent. These protein fractions, II and III, have the lowest content of lysine. The kafirins and alcohol soluble glutelins (fraction II plus fraction III) average about 51.2% in the normal sib as compared to 34% in P721 opaque. This reduction in fraction II and III accounts for part of the increase in lysine content of P721 opaque endosperm. In the fourth fraction which contains

Table 13. Percent distribution of endosperm, germ and pericarp of P721 opaque and normal.*

Sample	Endosperm	Germ	Pericarp
P721 opaque	81.9	11.3	6.9
Normal sib	84.9	9.8	5.3
Average of 4 sorghums**	84.2	10.1	6.0

* Average of two readings of about 200 seeds each.
** Jambunathan and Mertz (1973).

Table 14. Percent soluble nitrogen distribution in the P721 opaque and normal endosperm.*

Fraction	P721 opaque	Normal sib
I (saline)	11.6	7.1
II (isopropyl alcohol)	12.5	16.4
III (isopropyl alcohol + 2-mercaptoethanol)	19.5	31.8
IV (borate buffer + 2-mercaptoethanol)	5.4	6.5
V (borate buffer + 2-mercaptoethanol + sodium dodecyl sulfate)	45.0	32.2
Total Nitrogen extracted	94.0	94.1

* Average of two samples each.

nitrogen soluble in borate buffer and mercaptoethanol, there is a slight decrease in P721 opaque. The fifth fraction represents the glutelin fraction and the nitrogen in it is soluble in borate buffer, mercaptoethanol and sodium lauryl sulfate. This fraction increases in P721 opaque endosperm when compared with normal sib. Thus, the decrease in the percentage of soluble nitrogen in the second and third fractions in P721 opaque sorghum is compensated by a concomitant increase in the first and fifth fractions. The distribution pattern of P721 opaque are similar to the patterns observed in IS11758 (Jambunathan and Mertz, 1975).

Rat Feeding Experiment Comparing hl Sorghum IS 11758 with P721 Opaque

It is apparent from the rat feeding experiment in section IV that P721 opaque diet produced a three-fold increase in weight of weanling rats when compared to normal sib diet, as was the effect of high lysine sorghum diet of IS11758 (Singh and Axtell, 1973).

This experiment was conducted to compare the biological value of two high lysine sorghum lines IS11758 which is homozygous for (hl) gene and P721 opaque.

Materials and Methods

Selfed seed of IS11758 was available from the Puerto Rico harvest of 1973-74. P721 opaque and normal sib seed was from the increase at Purdue Agronomy Farm in 1974. As most of the selfed seed of P721 opaque was used up in the previous experiment (section IV) open pollinated seed of P721 opaque was mixed with remnant selfed seed to make up the diets for rats. RS610 control seed was from 1974 Lafayette harvest.

Experimental Procedure. Ten male weanling (21 days old) rats of Wistar strain (*Rattus rattus*) evaluated the growth response of each of the four sorghum diets, IS11758, RS610, P721 opaque and normal sib. ANRC casein-HCl diet was used for comparison. There was one rat in each cage and each rat was supplied water and food *ad libitum*.

Rats were arranged in groups of ten to make their average weight close to their overall mean weight and were placed in ten individual wire mesh cages. Grain samples were ground twice in a coffee grinder and analyzed for protein, fat, fibre and mineral content. All diets were brought to 10% protein level by the addition of corn starch. Oil and minerals, were added to bring the level to 5% and 4.5%, respectively. One percent of vitamin fortification mix was added to each diet. Protein and lysine contents were determined for the prepared diets. The other procedures and conditions were similar to the experiment in section IV.

Experimental Design. The experiment was considered as a completely random design. Data obtained were analyzed statistically, by the methods of Steel and Torrie, (1960).

Results and Discussion

Table 15 shows the chemical composition of whole grain samples and composition of rations as well as rat weight gain, feed efficiency ratio (FER), and protein efficiency ratio (PER). All grain rations were fed as isonitrogenous diets at approximately 10% protein. The average initial weight of weanling rats was 47.9 g. There was no significant difference in the average initial weight between groups of rats placed on different diets. Weight gain on the IS11758 and P721 opaque

Table 15. Biological values of isonitrogenous (10% protein) diets prepared with P721 opaque, IS11758 and normal sorghums in a 28-day rat feeding trial.

	Composition of grain	Composition of feed					
			Lysine				
Source	Protein percent	Protein percent	g/100 g protein	percent of sample	Weight gain (g) 28-days	FER	PER
IS11758	12.40	11.71	3.33	0.390	48.6	5.2	1.9
P721 opaque	12.41	11.8	2.83	0.334	31.8	6.8	1.5
normal sib	11.14	12.2	2.00	0.244	11.4	14.1	0.7
RS610	11.62	11.4	2.14	0.246	9.1	16.8	0.6
Casein	91.34	14.3	6.70	0.958	91.3	3.0	3.3
C.V., %					21.7		
S.E. of treatment mean					2.5		
LSD 5%					7.3		
LSD 1%					9.8		

was about 4.7 times and 3 times, respectively, more than that of the average of normal sorghums. Gain in weight of rats on P721 opaque ration was 65% of the gain on the IS11758 ration. However, it was significantly ($P<.01$) superior to the normal sorghum lines. The PER values for both high lysine sorghum lines were higher than the average PER for normal sorghum but were lower than that for casein.

Summary and Conclusions

Fractionation data on P721 opaque shows an increase in first and fifth fractions which are equivalent to albumin, globulin and glutelin fractions when compared to normal sib. There is a decrease in second and third fractions which represents alcohol soluble proteins (kafirins) and an increase in the fifth fraction which represents glutelins soluble in borate buffer, mercaptoethanol and sodium lauryl sulfate when compared to normal sib. The alcohol soluble fractions called the prolamines are known to have the lowest amount of lysine. The decrease in these fractions (II and III) and concomitant increase in first and fifth fractions are the reasons for increase in lysine content of P721 opaque.

When P721 opaque was compared to the best high lysine line IS11758 by feeding weanling rats diets at isonitrogenous (10% protein) levels, the average gain in weight of rats was 65% of the gain in IS11758 ration. It was still three times more than that of average of normal sorghums.

Comparison of Nutritional Value of High Lysine Grain Sorghum P721 Opaque and IS 11758 with Chicks

Featherston et al. (1975) have shown the nutritional superiority of high lysine sorghum IS11758 for the chicks. This study was conducted to compare the two high lysine sorghums IS11758, P721 opaque with commercial grain sorghum RS610 and the normal sib of P721.

The sorghum grain used in the preparation of diets for chicks was grown at Purdue Agronomy Farm during 1974 summer except for IS11758 which was grown at Puerto Rico in 1973-74 winter. Selfed seed was used in case of IS11758 but for P721 opaque, because of shortage of selfed seed, open pollinated seed was mixed with selfed seed to make up the rations.

Experimental Procedure

Day-old male White Mountain chicks (Gallus domesticus) were randomly allotted to treatments. Chicks were weighed, wing banded and placed in electrically heated chick brooders with raised wire floors. The heaters were set at about 96° F during the first week of the experiment. At the

* This experiment was performed at the Animal Science Dept., Purdue University, with Dr. W. R. Featherston and Dr. J. C. Rogler.

start of the second week the heaters were adjusted to 90° F, and this temperature was kept until the end of the experiment, when the birds were 18 days old. The room temperature was approximately 75° F. Each diet was fed to four replicate groups of seven chicks. No two replicates of the same treatment appeared in the same brooder or the same deck level of another brooder. Feed and water were provided ad libitum during the 18-day study. Normal sorghum (diets 1 and 2, Table 16) was compared on an isonitrogenous basis with high lysine sorghums (diets 3 and 4). Glucose monohydrate was added as the adjusting nutrient so that the diet would contain the proper amount of protein. In all treatments the level of protein in the diet was kept at 10.0%.

Chicks were randomly allotted to treatments. The treatments were arranged in 4 x 4 Latin-square design. Analysis of variance (Steel and Torrie, 1960) was used to statistically analyze final chick weight gain. Individual treatment differences were tested by the Newman-Keuls multiple range test (Steel and Torrie, 1960).

Results and Discussion

The four diets fed in the study contained only sorghum grain protein and all diets were isonitrogenous. Chicks fed the high lysine sorghum diet IS11758 and P721 opaque gained weight 2.8 and 2.1 times more than the average of normal sorghums and with about 50% and 40% less feed per unti weight gain than chicks fed the average of normal sorghum diets. As the diets were fed at isonitrogenous levels the greater gain in weight of chicks fed IS11758 diet when compared to P721 opaque diet is because of higher lysine content of the former. Gain in

Table 16. Composition of diets and the effect of sorghum grains RS610, IS11758 and P721 opaque on weight gain and feed conversion of chicks.

Ingredients	Diets (%)			
	1	2	3	4
Sorghum P721 normal sib	91.66	--	--	--
Sorghum RS610	--	90.17	--	--
Sorghum IS11758	--	--	85.08	--
Sorghum P721 opaque	--	--	--	82.50
Glucose monohydrate	--	1.49	6.58	9.16
Constant ingredients*	8.34	8.34	8.34	8.34
Total protein	10.09	10.09	10.09	10.09
Dietary lysine (g/100g protein)	1.98	1.81	3.10	2.74
Weight gain (g)**	13.4[a]	14.4[a]	39.0[b]	29.4[c]
Feed/gain ratio	5.67	5.45	3.53	3.84

* Constant ingredients provided (in %): dicalcium phosphate, 2.5; limestone, 1.0; soybean oil, 3.0; vitamin premix, 1.0; sodium chloride (iodized), 0.45; manganese sulfate, 0.05; zinc carbonate, 0.006; ferrous sulfate, 0.02; butylated hydroxytoluene, 0.125; choline chloride, 0.3; sodium selenite, 0.22 ppm.

** Mean values for 18 day-old chicks. Means bearing the same superscripts are not significantly different ($P>0.05$).

weight of chicks on P721 opaque rations was 75% of the gain on IS11758 diet with feed efficiency of 92% of IS11758.

Featherston et al. (1975) have already shown that improved performance of chicks fed the high lysine sorghum IS11758 diets as compared with chicks fed the normal sorghum grain diets was due solely to its higher lysine content. The improved performance of P721 opaque is due to similar reason, although definitive data is not yet available.

Summary and Conclusions

When sorghum grains were compared in diets in which all of the protein was supplied by the sorghum grain, at isonitrogenous levels (10.09% protein), the high lysine sorghums IS11758 and P721 opaque produced weight gains better than normal sorghum diet but IS11758 was somewhat superior to P721 opaque in weight gain of chicks as well as feed efficiency ratios.

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Studies on the Interferences of Tannins on Sorghum Grain Utilization by the Chick

Several different approaches have been used in recent studies to determine the mechanism by which sorghum grain tannins interfere with the utilization of the grain by the chick. These studies have utilized diets containing natural feedstuffs, such as sorghum grain and soybean meal, as well as purified components, such as crystalline amino acids and glucose, which should not require digestion prior to their absorption and utilization.

Results of these studies have often been difficult to explain. For example, a methanol extract from high tannin sorghum grain containing approximately 40% tannin equivalents does not have the same effect when added to chick diets as do the tannins in the grain itself. Whether the tannins have in some way been altered during extraction remains unknown. It is possible that the tannins have become more insoluble upon crystallization.

Previous studies have shown that methionine supplementation of a high tannin sorghum grain-soybean meal diet would alleviate much of the growth depression noted when high tannin sorghum grains are fed. In an attempt to further study the effect of methionine, chicks fed high and low tannin sorghum were orally dosed with one of two ^{14}C labelled forms of DL-methionine and the excreta collected for the following 24 hours. When expressed as a percent of the dose excreted in 24 hours, chicks fed the high tannin sorghum diet excreted approximately 1.5 times more radioactivity than chicks fed the low tannin sorghum diet regardless of whether the ^{14}C was located on the methyl group carbon or the number 3 carbon of the methionine molecule.

With chicks fed the same diet, approximately 2.5 times more radioactivity from the methyl group ^{14}C was excreted than from the number 3 carbon. Because the high tannin sorghum did not preferentially increase the excretion of radioactivity from the methyl carbon, it appears unlikely that methionine detroxifies sorghum tannins via methylation as has been proposed for tannic acid. The difference in excreted radioactivity between chicks fed the two diets was due to approximately 40% more excreta being produced by chicks fed the high tannin diets since on a per gram excreta basis, similar amounts of radioactivity were present. We plan to follow this study with a similar study using another ^{14}C labelled amino acid to see if the effects observed are specific for methionine or a general condition noted with all amino acids.

The studies in which methionine has been shown to overcome the detrimental effects of high tannin sorghum grain have been with diets supplemented with soybean meal which are first limiting in methionine for the chick. Studies were, therefore, conducted with sorghum grain-safflower meal-soybean meal diets which are first limiting in lysine and second limiting in methionine for the chick to see if methionine still produces a positive effect. Should such an effect be observed with this type of diet, which is not first limiting in methionine, it would be evidence that methionine is acting in some manner other than as an essential amino acid for protein synthesis.

The response noted to amino acid supplementation of the low tannin sorghum grain diets was similar to what might be expected. Methionine supplementation alone was ineffective, lysine supplementation produced a

marked improvement in weight gain and feed efficiency, and lysine + methionine supplementation resulted in a further improvement.

The response to amino acid supplementation of the high tannin sorghum grain diets is not as concise and easy to explain. First, unlike the results obtained with diets first limiting in methionine, the unsupplemented high tannin diet supported growth at a rate equal to the corresponding low tannin diet. Secondly, neither lysine nor methionine supplementation alone resulted in an improvement in chick performance. Supplementation with both lysine and methionine together resulted in a significant improvement in weight gain and feed efficiciency of the chicks but not to the same level as noted with the low tannin grain diets. This same pattern of results was consistently reproduced in a second experiment.

In a preference trial with four-week-old chicks given a choice of low tannin RS610 sorghum or high tannin BR64 sorghum, chicks consumed an average of 31.7 grams of low tannin sorghum and 3.1 grams of high tannin sorghum on days 2-5 of the trial, or approximately 10 times more low tannin grain when fed as the whole kernel. When the grain was ground and fed to the chicks on days 6-9 of the trial, an average of 34.1 grams of low tannin and 6.9 grams of high tannin grain were consumed, or approximately five times more low tannin grain. These results indicate that the high tannin grain is discriminated against by chickens as has been observed with wild birds in the field and that maturation, storage, and grinding do not overcome this discrimination. The reason for the preference of low tannin sorghum is puzzling since it is very unlikely that the chick can discern any stringent taste of tannin in the whole kernel.

E. DISSEMINATION AND UTILIZATION OF RESEARCH RESULTS

1. Dissemination of Results.

a. Research reports.

Copies of the 1973 research report were sent to cooperators in Pakistan, Denmark, India, Kuwait and Egypt, in addition to the approximately 800 copies previously distributed in 109 states and countries.

The Second International Grain Sorghum Protein Yield and Quality Trial, Report No. 2, Station Bulletin No. 125 was distributed to all cooperators for their use in selecting broadly adapted high yielding entries as germplasm sources for more localized breeding programs.

b. Breeder samples of high lysine genotypes.

In addition to requests for seed of IS 11758 and IS 11167 filled in 1973 and 1974, samples of these high lysine lines were supplied to an additional 46 cooperators in 21 countries and seven states in 1975. The number of workers receiving seed samples were as follows: Peru (1), Nicaragua (3), Brazil (2), Mexico (7), El Salvador (1), Virgin Islands (1), Puerto Rico (3), Venezuela (3), Panama (1), Dominican Republic (1), Argentina (2), Haiti (1), Sudan (1), Niger (1), Upper Volta (1), Egypt (1), Cameroon (1), Italy (2), Japan (1), India (1), Australia (2), Romania (1), Maryland (1), Texas (2), California (2), Arizona (1), Louisiana (1), Illinois (1), and Washington, D. C. (1).

Breeder samples of the chemically induced opaque high lysine mutant P721 were first distributed in 1974. In 1975, seed of this lines was supplied to an additional 77 cooperators representing 30 countries and

17 states as follows: Peru (1), Brazil (3), Mexico (5), Puerto Rico (3), Venezuela (2), Guatemala (1), Dominican Republic (1), El Salvador (1), Nicaragua (1), Ecuador (1), Haiti (1), Honduras (1), Sudan (1), Niger (1), Upper Volta (1), Egypt (1), Cameroon (1), Hungary (1), Italy (2), Romania (1), Israel (1), Japan (1), Thailand (1), Indonesia (1), Viet Nam (1), Australia (2), Hawaii (1), Illinois (3), Washington, D. C. (1), New Hampshire (1), Texas (12), Nebraska (2), Iowa (2), Kansas (3), Missouri (1), Maryland (1), California (1), Indiana (1), Pennsylvania (1), Ohio (1), Mississippi (1), Louisiana (1), and Georgia (1).

c. High lysine seed distribution for research purposes.

Larger quantities of high lysine seed were supplied other scientists conducting research related to nutrition, cereal technology, grain quality, and physiology. Included were the following: Adamson/Washington, D. C.; Wu/Illinois; Landi/Italy; Schaffert/Brazil; Reddi/India; and Clark/Texas.

d. Seminars and papers presented.

Axtell, J. D. "Nutritional Quality Research in Sorghum" Sigma Xi, Purdue Chapter, April 28, 1975.

Featherston, W. R., "Nutritional Value of High Lysine Sorghum Grains for the Chick". Poultry Science Association meetings, Pullman, Washington. July 31 - August 10, 1975

Porter, K. S. "Laboratory and In Vitro Evaluation of Forage from Chemically Induced Brown Midrib (bmr) Mutants of Sorghum bicolor (L.) Moench". American Society of Agronomy Meetings, Knoxville, Tennessee, August 25-29, 1975.

Mohan, D. P. "Inheritance of High Lysine Character of Grain Sorghum Opaque Mutant P721, Its Biological Evaluation and the Structure of its Endosperm." American Society of Agronomy, Knoxville, Tennessee, August 25-29, 1975.

Axtell, J. D. "Progress Report on Nutritional Quality Improvement in Sorghum". AID Review Meetings, Lincoln, Nebraska, September 14-17, 1975.

Featherston, W. R. "Detrimental Factors in Corn and Sorghum Grain for Poultry". Purdue Feed Industry Conference. Purdue University, W. Lafayette, Indiana. October 6, 1975.

Axtell, J. D. "Naturally Occurring and Induced Genotypes of High Lysine Sorghum. IAEA Meetings, Vienna Austria, May 5-9, 1975.

Featherston, W. R. "Interference of Tannins on Sorghum Grain Utilization by Monogastric Animals". Animal Nutrition Research Council, Washington, D. C. October 16, 1975.

e. Selection germplasm utilization.

The Second International Grain Sorghum Protein Yield and Quality Trials have assisted cooperators in identifying germplasm suitable for either immediate release or for inclusion in breeding programs. Of the 24 entries distributed, 11 were selected by one or more cooperators for potential use either directly or for breeding purposes. Those lines selected were as follows: IS 2375, 954066, 954052, 954054, 954035, IS 8309, IS 3198, IS 9958, IS 3441, and IS 7822.

Results from the Third International Yield and Quality Trial have been returned and are being summarized at the present time. Preliminary data again show a wide range in adaptability and yield potential among the lines included.

2. Evidence and Cases Where Findings are Being Used in LDC's and the U.S.

Contact with sorghum workers internationally continued to be an important link in the dissemination utilization process. Germplasm or information exchange occurred with sorghum workers as follows:

	No. of countries or states	No. of workers
Africa	12	16
Asia	10	22
Australia (area)	2	5
Europe	7	9
N. America	29	84
S. America	8	14
	68	150

Germplasm supplied by this project continued to provide useful material from which lines adapted to specific areas may be selected. Leake/Senegal selected 954063 and 954206 as promising lines based on yield and agronomic

characteristics. Winson/Niger selected the same two lines, plus 954164, 932027, 932075 and 954130. Moore/Bangladesh selected IS 2941, IS 12282, 498031 and 498023.

Requests for seed of specific lines for specific areas or purposes were answered for Atkins, Iowa, Eastin/Nebraska, Garrison/Maryland, Parodi/Argentina, Menge/Minnesota, Hinze/Colorado, Robison/Nebraska, Marathee/France, Tarumoto/Japan, Singhania/India, Kabore/Upper Volta, Gross/Minnesota, Haslem/United Kingdom, Brindley-Richards/Rhodesia, Schaffert/Brazil, Clark/Texas, Youngs/Canada, Hanna/Georgia, Harris/Georgia, Curtis/Iowa, Kirleis/Indiana, Malenga/Malawi, Sebatutsi/Burundi, Singh/CIMMYT-Mexico, Ford/Cameroon, Judy/Pakistan, Leger/Senegal, House/ALAD-Lebanon, and Doggett/ICRISAT-India. This accounts for 16 countries, 8 states, and 29 individuals.

Specific examples illustrating the kind of requests and assistance given follow:

From: N.E.A. Malenga - Ngabu, Malawi - January 6, 1976

> For the past three years personnel of the Shire Valley Agricultural Development Project have been evaluating a number of sorghum varieties both exotic and local, good for human consumption in Malawi. Among these lines, two have been traced to have originated from the Purdue International Protein Yield Trials. They are identified in Malawi as PN3 and PN8. Performance of both PN3 and PN8 has consistently topped the list for the three-year program and PN8 is running superior to PN3. They have recommended these lines to be grown nationally and would like to carry out an expanded seed multiplication program immediately.

From: F. Leger - Dakar, Senegal - March, 10, 1976

Two entries received from the Purdue-AID Sorghum Project (954063 and 954206) were forwarded by Leger to CNRA/Bambey; IRAT/Upper Volta; CNRADA/Mauritania; IRAT/Mali; INRAN/Niger for observation plots to be included in the "1976 West African Short-Season Uniform Sorghum Trial". (If another yield trial was available he wanted to send it to Cabo-Verde Republic, Nigeria, Northern Togo, Senegal, The Gambia, Mauritania, Mali, Upper Volta, Niger, Nigeria, Northern Dahomey, Cameroon, Chad, and Central African Republic).

From: K. Oyeleke - Zaria, Nigeria - January 10, 1976

Requested all information, reprints, publications from this project to assist him as a staff member in the Biochemistry Department of Ahmadu Bello University, as they focus on assessing the protein quality of locally grown sorghum varieties and look into the effect of various cooking methods and preparation procedures on the protein quality of sorghum, also assessing the amino acid analyses.

From: T. N. Khan - Port Moresby, New Guinea - June 1975

Starting a sorghum and maize breeding program funded by IDRC Canada, Mr. Khan is requesting Purdue-AID Sorghum Project assistance in supplying genetic material.

From: M. Ford - American Embassy/Cameroon - March 2, 1976

Managing a sorghum seed multiplication project for USAID program in Cameroon, Mr. Ford is requesting seed for field trials in the semi-arid region of N. Cameroon.

From: E. Sebatutsi - Bujumbura,Burundi - February 1976
Representing the Institut des Sciences Agronomiques du Burundi, he wishes to introduce new sorghum varieties to Burundi, and is requesting IS 3487, IS 3462, IS 2410, IS 3548, and IS 8965.

From: H. V. Arkel - Nakuru, Kenya - October 1975
The Kenya Beef Industry Development Project is involved in the introduction and popularization of sorghum in Kenya and is a relatively new crop to the Kenya highlands, and there is little known about the introduced varieties which proved to be able to yield substantial amount of grain, and which all originate in the high altitude areas in Uganda and Ethiopia. The project is about to be involved in a large-scale cultivation program aimed at production of grain for the feed milling industry. With no facilities to evaluate grain quality in terms of tannin content and amino acid spectrum, they are seeking help from the Purdue-AID Sorghum Project.

In addition, analyses were performed for cooperators in India, Kenya, Austria, Egypt, and Puerto Rico, and technical information regarding methods or equipment was provided to cooperators in Mexico, Thailand, Denmark, Egypt, Cameroon, India, Kuwait, Argentina, and Senegal, as well as workers in the U.S.

3. Plans to Use Research Data More Effectively.

Continued training of persons from LDC's is being utilized to provide research workers with emphasis on protein quality for national sorghum programs.

Cooperative efforts and sharing of germplasm and data continue with international centers (ICRISAT, ALAD, and CIMMYT) and with the sorghum research workers in Puerto Rico, Texas, and Nebraska.

4. Involvement of LDC or US Personnel and Institutions.

Students from Lebanon (Vartan Guiragossian on a Rockefeller Fellowship), Ethiopia (Gebisa Ejeta) and United States (Paul Christensen with Peace Corps experience in Ethiopia, Steve Van Scoyoc and K. S. Porter) continue graduate training during 1975. Students from India (D. P. Mohan) and Brazil (Raimundo de Pontes Nunes) completed Ph.D. degrees with the project in 1975.

Correspondence dealing with the high lysine releases, tannin research, germplasm for developing breeding programs, and other closely related areas was extensive and provided increased contact and fostered improved cooperation with commercial, national, state, and local sorghum research projects in both the LDC's and the U.S.

The project will be represented at an East African Cereals Conference in Tanzania in 1976. Gebisa Ejeta will present a paper authored by him and Dr. Axtell summarizing nutritional quality research in sorghum.

The project has provided germplasm and information regarding the high lysine lines, as well as other material to the international centers (ICRISAT, ALAD, and CIMMYT) to expand the opportunity for development of locally adapted high lysine types for LDC's in the respective areas.

In addition, germplasm and/or data have been supplied directly through correspondence as follows: Australia (5), New Guinea (1), Kenya (1), Rhodesia (2), Egypt (2), Cameroon (2), Kuwait (2), Senegal (3), Niger (1), Tanzania (1), Ivory Coast (1), Malawi (1), Nigeria (1), Burundi (1), Pakistan (2), India (20), Thailand (2), Bangladesh (2), Lebanon (2), Yeman (1), Philippines (2), Japan (2), Indonesia (2), Viet Nam (1), Austria (5),

Romania (1), United Kingdom (1), Italy (1), Denmark (2), France (1), Hungary (1), Puerto Rico (6), Brazil (2), Paraguay (1), Bahamas (3), Venezuela (2), Argentina (2), Honduras (1), Chile (1), Canada (4), Mexico (6), and the U.S. (104). This represents overseas correspondence with 42 countries, as well as extensive correspondence in the U.S.

The project and its personnel have hosted visitors from many areas of the world including the following: Gebrekidan/Ethiopia, Judy/Pakistan, Worzella/Wisconsin, Rosenfield/Indiana, Gomez/Philippines, Baka/Illinois, Rojas/ICA-Colombia, Gavva/USSR, Short/Ohio, Fernandes/Brazil, Sanon/Upper Volta, Kabore/Upper Volta, Solh/Lebanon, Nasr/Lebanon, Sherma/Alabama, Shuford/Alabama, Done/Australia, Kebede/Ethiopia, House/ALAD-Lebanon, and Eastin/Nebraska. This represents visitors from 15 countries or states. Such visits result in exchange of information and increased cooperation.

Bibliographic List and Short Abstracts of Research Reports for 1975 Representing Efforts to Disseminate the Results of the Research Project.

Featherston, W. R. and J. C. Rogler, 1975. Influence of tannins on the utilization of sorghum grain by rats and chicks. Nutrition Reports Int'l. Vol. II No. 6:491-497.

Featherston, W. R., J. C. Rogler, J. D. Axtell, and D. L. Oswalt. 1975. Nutritional value of the high lysine sorghum grain for the chick. Poul. Sci. 54:1220-1225.

Mohan, D. P., J. D. Axtell, E. T. Mertz, W. R. Featherston, and J. C. Rogler. 1975. Inheritance of high lysine character of grain sorghum opaque mutant P721, its biological evaluation and the structure of its endosperm. Agron. Abs. p. 59.

Porter, K. S., V. L. Lechtenberg, and J. D. Axtell. 1975. Laboratory and *in vitro* evaluation of forage from chemically induced brown midrib (*bmr*) mutants of *sorghum bicolor* (L.) Moench. Agron. Abs. p. 109.

Mohan, D. P. 1975. Chemically induced high lysine mutants in Sorghum bicolor (L.) Moench. Ph.D. Thesis, Purdue University.

Nunes, R. 1976. Genetic variation and covariation in hybrids of new restorer and cytoplasmic male sterile lines of sorghum from the world collection. Ph.D. Thesis, Purdue University.

Porter, K. S. 1975. The inheritance, fiber composition and in vitro digestibility of chemically induced brown midrib mutants of Sorghum bicolor (L.) Moench. M.S. Thesis, Purdue University.

Clark, J. 1975. Three screening procedures for estimating protein quality in Sorghum bicolor (L.) Moench grain. M.S. Thesis, Purdue University.

Axtell, J. D. 1976. Naturally occurring and induced genotypes of high lysine sorghum. Proceedings of Conference on "Evaluation of Seed Protein Alterations by Mutant Breeding". International Atomic Energy Agency. Vienna, Austria pp. 45-53. (Meetings in Hahnenklee, W. Germany)

ABSTRACT

INFLUENCE OF TANNINS ON THE UTILIZATION OF SORGHUM GRAIN BY RATS AND CHICKS. W. R. Featherston and J. C. Rogler

Extraction of the tannins from a high-tannin (bird-resistant, BR) sorghum grain resulted in marked improvements in weight gain and feed efficiency of rats as compared with rats fed the intact unextracted grain. Protein and energy digestibility values were significantly reduced for rats fed the BR sorghum diet as compared with rats fed the extracted BR sorghum or a low-tannin sorghum grain diet. Supplementation of the BR sorghum diets with L-methionine or the hydroxy analogue of methionine did not alleviate the detrimental effects of the dietary tannins in rats, whereas chick growth and feed efficiency were significantly improved with similar supplementation. The magnitude of the response to methionine supplementation was much greater with chicks fed BR sorghum than with those fed a low-tannin sorghum.

Nutrition Reports Int'l
Vol. II no. 6 pp. 491-497

ABSTRACT

NUTRITIONAL VALUE OF HIGH LYSINE SORGHUM GRAIN FOR THE CHICK.
W. R. Featherston, J. C. Rogler, J. D. Axtell, and D. L. Oswalt

Growth studies were conducted with day-old chicks to compare the nutritional quality of a high lysine sorghum grain (IS11758) with two low-tannin commercial sorghum grains (RS671 and RS610) in diets containing various protein levels. When the sorghum grains were compared on either an equal weight or equal protein level in diets in which all the protein was supplied by the sorghum grains, the high lysine sorghum produced weight gains of approximately three times those noted with the commercial ("normal") sorghum and with approximately 50% less feed required per unit gain. Supplementation of the normal sorghum diet with lysine to equal the level supplied by the high lysine sorghum resulted in similar chick growth and feed efficiency. These results indicate that the improved performance of chicks fed high lysine sorghum was due solely to its higher lysine content.

Chicks fed the high lysine sorghum diets supplemented with safflower meal or soybean meal to a 15% protein level grew 2.5 and 1.9 times faster, respectively, and were significantly more efficient in feed utilization than their corresponding counterparts fed normal sorghum diets.

These results indicate that the nutritional quality of high lysine sorghum grain is markedly superior to that of two commercial sorghum grains.

Poultry Science 54:1220-1225, 1975

ABSTRACT

INHERITANCE OF HIGH LYSINE CHARACTER OF GRAIN SORGHUM OPAQUE MUTANT P721, ITS BIOLOGICAL EVALUATION AND THE STRUCTURE OF ITS ENDOSPERM.
D. P. Mohan, J. D. Axtell, E. T. Mertz, W. R. Featherston, and J. C. Rogler.

A diethyl sulfate induced high lysine opaque mutant (P721) in sorghum has been inherited as a partial dominant. The nutritional quality of rations prepared from P721 opaque seed is better than the normal sorghum. When these rations are fed at isonitrogenous levels (10% protein) to weanling rats, gain in weight is three times more than when they are fed rations prepared from normal sorghum grain. Isonitrogenous rations (10% protein) of the best Ethiopian high lysine line from the world collection (IS11758) and the P721 opaque were compared in feeding trials with weanling rats and chicks. IS 11758 was found to be superior. Gain in weight of rats and chicks on P721 opaque ration was 65% and 75% of the gain on IS 11758 ration, respectively. No protein bodies were visible in P721 opaque while they were abundant in the normal sib in endosperm sections under the light microscope.

Agronomy Abstracts, 1975
p. 59

ABSTRACT

LABORATORY AND *IN VITRO* EVALUATION OF FORAGE FROM CHEMICALLY INDUCED BROWN MIDRIB (*bmr*) MUTANTS OF *SORGHUM BICOLOR* (L.) MOENCH.
K. S. Porter, V. L. Lechtenberg, and J. D. Axtell

Seeds of two sorghum lines were treated with diethyl sulfate (1 and 2 ml DES/1000ml H_2O/3 hours) to induce mutations. Nineteen induced brown midrib (*bmr*) mutants were identified in M_3 head rows segregating for the *bmr* character. Thirteen mutants and their normal counterparts were evaluated in the laboratory for percent cell wall constituents, acid detergent fiber, lignin, cellulose and hemicellulose, and for percent *in vitro* dry matter disappearance (IVDMD) and *in vitro* cell wall constituent disappearance (IVCWCD). The *bmr* genes acted primarily to reduce lignin content. Reductions in lignin among mutants ranged from 9-44% in stem tissue. Other fiber components were altered less. Reductions in percent lignin in stem tissue resulted in increases ranging from 4-18% for IVDMD and from 0-43% for IVCWCD. Differences among mutants were observed suggesting the presence of several different *bmr* genes although allelism tests have not been completed.

Agronomy Abstracts, 1975
p.109

ABSTRACT

CHEMICALLY INDUCED HIGH LYSINE MUTANTS IN SORGHUM BICOLOR (L.) MOENCH.
D. P. Mohan.

The parental lines used in the mutagen treatments had the following characteristics: (1) photoperiod insensitivity, (2) 3-dwarf, (3), broad agronomic adaptibility, (4) colorless pericarp, and (5) translucent endosperm. Selfed seed was treated with 0.1% diethyl sulfate (DES) for three hours to induce opaque endosperm mutants at the rate of 75 g seeds/ 1000 ml water.

Four hundred seventy putative opaque mutants were selected by screening over a light box for opaque mutant seeds from 23,000 M_2 heads (bearing M_3 seeds) grown in Puerto Rico. Normal (vitreous) and opaque seeds were separated from each segregating M_2 head and samples from each seed class were analyzed separately. Thirty-five opaque endosperm mutants had an increase in lysine concentration of at least 50%. When these high lysine opaque seeds were planted some grew into normal looking plants (Class I), but most were abnormal showing pleiotropic sphorophytic effects ranging from complete lethality to dwarfs and chlorophyll deficiencies or female or male sterility (Class II).

One high lysine opaque mutant (P721 opaque) belonging to Class I had an average (over two locations) whole grain lysine content of 2.9 (g/100 g protein) at 15.7% protein relative to normal sib average of 1.9% lysine at 14.2% protein.

The inheritance data suggest that the opaque character is simply inherited as a partial dominant. A preliminary test for allelism indicates that this gene is nonallelic with the (hl) gene in IS 11758.

The effect of the high lysine gene is seen in the changed amino acid

pattern. There is an increase in lysine and arginine content and a decrease in glutamic acid, alanine, and leucine content in opaque seeds relative to normal vitreous sib seeds. Fractionation studies indicate that there is a decrease in alcohol soluble proteins (prolamines) and concomitant increases in other fractions which are more desirable nutritionally.

The average test weight of P721 opaque seeds is less by 25% than the normal sib seed.

The nutritional quality of rations prepared from P721 opaque seed is better than the normal sorghum. When these rations are fed at isonitrogenous levels (10% protein) to weanling rats, gain in weight is three times more than when they are fed rations prepared from normal sorghum grain.

Isonitrogenous rations (10% protein) of the best Ethiopian high lysine line from the world collection (IS 11758), and the P721 opaque were compared in feeding trials with weanling rats and chicks. IS 11758 was found to be superior. Gain in weight of rats and chicks on the P721 opaque ration was 65% and 75% of the gain on IS 11758 ration, respectively.

No protein bodies are visible in P721 opaque while they are abundant in the normal sib in endosperm sections under the light microscope.

The P721 opaque possess many agronomic characteristics which should make it useful in the improvement of sorghum lines and hybrids. It is photoperiod insensitive, short enough to allow combine harvesting, and has wide agronomic adaptability. The seed of P721 is plump with a white pericarp, but of greatest importance is the high lysine content coupled with the low isoleucine-leucine ratio. The P721 opaque should prove valuable in both temperate and tropical regions of the world.

Ph.D. thesis
Purdue University

ABSTRACT

GENETIC VARIATION AND COVARIATION IN HYBRIDS OF NEW RESTORER AND CYTOPLASMIC MALE STERILE LINES OF SORGHUM FROM THE WORLD COLLECTION.
R. Nunes

Genetic variation was investigated among 16 new restorer lines (R-lines) selected from the world sorghum collection, and one chemically induced mutant and its normal sib, used as male parents, and 14 newly developed cytoplasmic male sterile lines (A-lines) and four standard commercial A-lines, used as female parents.

The 18 R-lines were divided in two groups. The 16 lines from the world sorghum collection were first randomly subdivided into two groups of eight. The two remaining R-lines were added to each of the two groups of eight so that each of the two groups of males was composed of 10 lines. The eight new R-lines were referred to as random males because they were regarded as a random sample from a much larger number of phenotypically desirable R-lines that could be identified in the world sorghum collection. The induced mutant and its normal sib were called fixed males.

Similarly, the 14 new A-lines were divided at random into two groups of seven. The four standard male sterile lines were added to each of the two groups so that both groups of females had 11 lines. The 14 newly developed A-lines were called random females because they were regarded as a random sample from a much larger number of phenotypically desirable A-lines that could be developed from the world sorghum collection. The four standard A-lines were called fixed females because they were deliberately chosen.

Each of the two groups of males was matched at random with one of the two groups of females. As a result, two "AB" overall mating designs were

formed and since there were lines which were random and lines which were fixed in each mating design, each was subdivided into four sub-designs which were referred to as: model 1 - random males by random females; model 2 - random males by fixed females; model 3 - fixed males by random females and model 4 - fixed males by fixed females. Four different statistical models were used to describe the progenies within each sub-design.

The characters investigated were seedling vigor, days to flower, plant height, threshing percent, panicle weight, kernel weight, dye binding capacity, protein percent, lysine percent, number of panicles per hectare and grain yield. Means for catechin equivalents were also presented.

The variation due to random males was significant for days to flower, plant height, and kernel weight in model 1 and for all characters except panicles per hectare in model 2.

In model 3 the fixed males were significant for DBC and protein percent and in model 4 males were nonsignificant for all characters except kernel weight.

Random females were significant for seedling vigor, days to flower, plant height, kernel weight, and DBC in model 1 and for all characters except seedling vigor and threshing percent in model 3.

In model 2 the fixed females were significant for days to flower, DBC, and threshing percent, and in model 4 those fixed females were nonsignificant for all characters.

The male by female interaction was significant for all characters except DBC and threshing percent in model 1, for days to flower, plant height, panicle weight, kernel weight, and grain yield in model 2, for plant height, panicle weight, and yield in model 3, and nonsignificant for all characters in model 4.

Variance component estimates for males of models 1 and 2 were greater than the estimates involving interactions with location, the relative magnitude of these differences varying with characters.

Variance components for females of models 1 and 3 were greater for certain characters than those estimated for males in model 1 and greater than the estimates of the interaction component for location x males x females in both models. The magnitude of these differences varies with different characters.

Significant or highly significant phenotypic and genetic correlation coefficients were found among different pairs of variables for random males and/or random females of models 1, 2 and 3. No correlations were estimated for model 4.

General combining ability (GCA) estimates varied considerably among all parent lines in all four models and in relation to each character. A common feature of this particular material was that all parent lines presented combinations of positive and negative GCA values relative to different characters suggesting genotypic balance toward fitness. Some combinations such as positive values for grain yield and negative values for days to flower and plant height that are considered agronomically desirable were found.

Specific combining ability was found to be very important for yield, and in this respect it varied widely. Model 3 presented the highest yielding specific male x female combinations and also some of the lowest. Grain yield ranged from 12696 kg/ha to 3595 kg/ha in this model. Further research to evaluate the possibilities of using some of these new hybrids in commercial production is strongly recommended.

Ph.D. thesis, 1976
Purdue University

ABSTRACT

THE INHERITANCE, FIBER COMPOSITION AND *IN VITRO* DIGESTIBILITY OF CHEMICALLY INDUCED BROWN MIDRIB MUTANTS OF *SORGHUM BICOLOR* (L.) MOENCH.
K. S. Porter.

Nineteen brown midrib (*bmr*) mutants were induced by treating seed of two sorghum lines (954104 and 954114) with diethyl sulfate (DES). Mutants were isolated from M_3 head rows segregating for the brown midrib character and were designated *bmr*-1 through *bmr*-19 with normal sister segregates being designated N-1 through N-19.

Mutant plants showed characteristic brown pigmentation in the leaf midrib, particularly on the abaxial side of the leaf, and in the stem behind the leaf sheath. Pigmentation was also present in the culm, the immature panicle branches and in the stem pith of mature plants. Pigmentation became distinct in the 4-6 leaf stage but varied in intensity among mutants and tended to fade as plants matured. Mutants varied greatly in plant phenotype and fertility and of the original 19 isolated, 6 were discarded due to sterility and poor phenotypic expression of the brown midrib character.

Inheritance data on 8 of the mutants showed the brown midrib character to be conditioned by simple recessive genes. It is probable that several different genes were present in the group evaluated and allelism tests are needed to determine which mutants are allelic.

Stem and leaf tissue of each mutant and its corresponding normal counterpart was evaluated in the laboratory for percent cell wall constituents (CWC), acid detergent fiber (ADF), permanganate lignin (PL), cellulose, hemicellulose, *in vitro* dry matter disappearance (IVDMD) and for percent *in vitro* cell wall constituent disappearance (IVCWCD). Laboratory data showed no significant differences in percent CWC of stems of normal and mutant plants in 11 of the 13 pairs evaluated. Leaf tissue in general showed fewer differences than

stem tissue due to the reduced fiber content of leaves. Significant reductions in percent ADF occurred in four mutants but were primarily due to reductions in percent cellulose in the same mutants. Reductions in cellulose content were greater than expected on the basis of work with brown midrib maize and must be due to slightly different gene action in sorghum. Changes in percent hemicellulose were insigifnicant and showed no consistent trends.

Of the fiber components evaluated, lignin was most closely associated with the bmr gene. Mutants had significantly less lignin than normal plants in 10 of the 13 comparisons. Reductions in lignin ranged from 9% - 44% in stems and from 5% to 25% in leaves.

Significant increases in percent IVDMD, which occurred in 10 of the 13 pairs, corresponded in 9 of 10 cases with significant reductions in percent lignin. Of the fiber components considered, lignin showed the largest negative correlation (-.79 with percent IVDMD). Regression analysis showed an average 3.3% increase in IVDMD to be associated with a 1% decrease in lignin. Increases in IVDMD of stems averaged 6.7 percentage units across all pairs.

Increases in IVCWCD percentages were consistently associated with the presence of the bmr gene and reductions in lignin. Lignin content showed a large negative correlation (-.75) with percent IVCWCD. Cellulose and hemicellulose showed smaller negative correlations (-.39, -.09). Regression analysis showed a 3.09% and 2.30% increase in IVCWCD for each 1% decrease in lignin of stems and leaves.

The influence of the bmr gene on certain agronomic characteristics was studied to determine its potential usefulness. The gene was found to have little affect on maturity as measured by days to 50% flower. Height was influenced

to a greater extent with brown midrib plants in some cases being slightly shorter. Yield data, due to early frost, was inconclusive and must be evaluated in a more favorable year. Other factors needing evaluation are disease and insect resistance and lodging susceptability.

The mutants bmr-2, bmr-6, bmr-12, bmr-17 and bmr-18 appeared to be the most promising in terms of future research for forage quality improvement. The bmr gene in sorghum can be a powerful tool in manipulating one of the factors (lignin) limiting intake, digestibility, feed efficiency, and total animal performance.

M.S. Thesis, 1975
Purdue University

ABSTRACT

THREE SCREENING PROCEDURES FOR ESTIMATING PROTEIN QUALITY IN *SORGHUM BICOLOR* (L.) MOENCH GRAIN.
John Clark.

Three screening methods for improving protein quality in grain sorghum were examined. In the first study, 14,000 M_2 and 400 M_3 grain sorghum samples from a diethyl sulfate mutagenic treatment were analyzed for protein quality by the dye binding method using the NEOTEC Grain Quality Analyzer for an independent nitrogen determination. Forty M_3 heads were selected for protein and lysine determination. Of these selections, 38 were found to be high in protein, but low in lysine (averaging 16.6% protein and 1.80 g lysine/ 100 g protein compared to values of 13.2% and 2.10 g for the untreated line). However, two M_3 sibs, 51016-1 and 51016-2 had approximately a 30% increase in lysine percent of sample over the parent line and a 40% increase over average sorghum. Mutant 51016 also had increases in percent protein, percent oil, and percent amylose. The increases in lysine, oil, and amylose of 51016 appear to be inherited as a simple recessive character. Mutant 51016 is a colorless pericarp, partially vitreous endosperm sorghum. Although this mutant has less lysine and protein than the high lysine lines reported by Singh and Axtell (1973), it is neither dented, nor completely floury, and, therefore, has a more desirable kernel phenotype. Based on regression equations relating DBC with protein, and protein with lysine concentration, the dye binding method appears to be an effective screening procedure for estimating lysine levels when used in conjunction with an independent estimate of protein.

A second study was conducted to determine if modifiers of the high lysine (hl) gene could be found to alter the dented, high lysine phenotype to a more plump and acceptable kernel shape, while retaining the high lysine levels. Thirteen heads from Purdue University random mating grain sorghum populations segregating for the hl gene were selected on the basis of a plump-opaque kernel phenotype. Seed from each selected head was sorted into opaque and vitreous classes, and both classes analyzed for protein and lysine. The opaque kernels were completely opaque or partially opaque (1/2 opaque:1/2 translucent). Several of the selected heads contained approximately three normal seeds to one opaque seed, suggesting a simple recessive gene for the opaque character. However, none of the plump-opaque seeds were significantly higher in protein or lysine than the normal vitreous class. The opaque classes are, therefore, believed to be a consequence of floury genes unrelated to the hl gene or to immaturity effects and not to modified hl expression.

A third study describes the subcellular protein structure of normal and high lysine sorghum. The endosperm structure of high lysine sorghum was distinctly different from that of floury and vitreous kernels with normal lysine concentrations. Although a few protein bodies were observed in high lysine endosperm, there were far less than the number found in normal endosperm. A modified high lysine expression (partially vitreous) had some characteristics of both high lysine and normal kernels. The use of microscopic examination of endosperm as a selection tool for protein quality is discussed.

M.S. Thesis, 1975
Purdue University

ABSTRACT

NATURALLY OCCURRING AND INDUCED GENOTYPES OF HIGH LYSINE SORGHUM.
J. D. Axtell

The discovery of a high lysine genotype of grain sorghum from a natural population and the identification of a high lysine mutant in a mutagenized population is described. Chemical, genetic, and other biological characteristics of the two differently derived high lysine germplasm types are described. Preliminary results suggest that the protein quality of both sources of high lysine sorghum germplasm is the same. The factors influencing the biological value of the sorghum grain are discussed briefly, including not only lysine content, but also tannin content. A discussion of prolamine protein inheritance in grain is presented with some suggestions for research.

Proceedings, Evaluation of Seed
Protein Alterations by Mutation
Breeding. International Atomic
Energy Agency. Vienna, Austria
pp. 45-53. 1976.

F. STATEMENT OF EXPENDITURES AND OBLIGATIONS AND CONTRACTOR RESOURCES

Category:	Total Adjusted Budget Amount 4/7/75 - 3/31/76	Expenditures 4/1/75 - 3/31/76
Salary and Wages	$132,500	$132,216.59
Salary Related Costs	10,800	10,540.82
Overhead	90,763	90,568.37
Travel	8,500	7,841.75
Other Direct Costs (Computing/ Publications)	3,300	3,363.26
Equipment, Materials & Supplies	30,577	40,442.10
Subcontracts & Nurseries	22,538	14,005.11
Consultants	1,000	1,000.00
	299,978	299,978.00

As of 9/30/76

G. WORK PLANS FOR 1976-77

Objective 1 - Identify and evaluate sorghum lines or mutants with improved nutritional quality using both chemical and biological methods.

a. Chemical analysis of new world collection sorghum lines for tannin, lysine, and protein content will continue. This will include new germplasm from Cameroon and Ethiopia, and other sources.

- Tannin content will be estimated as catechin equivalents which has been a reliable assay that is consistently negatively correlated with rat response.
- Both high and low tannin sorghum lines will be screened for lysine content using the dye binding technique followed by rat feeding trials on the best entries.

b. Evaluate M_3 progeny of high lysine opaque (P721) lines treated with chemical mutagens in 1947-75 for the identification of vitreous endosperm mutants (su_2 types).

c. Evaluate M_4 progeny of high lysine opaque (P721) lines from chemical mutagen treatments by using dye binding capacity to identify mutants which further reduce the prolamine content.

d. Following identification of 500 opaque endosperm mutants from chemical mutagen treatments in 1972, the contractor shall continue research on on other potentially useful high lysine mutants using progeny testing,

chemical analyses and biological evaluation.

e. Determine the feasibility of screening for mutants or lines in the world collection which have alterations in the normal feedback inhibition control mechanism for lysine biosynthesis. These mutants are known to overproduce lysine in other higher plant cell systems. This provides a mechanism to use the genetic machinery of the cell to "fortify" the sorghum grain with lysine without reducing prolamine synthesis. Screening techniques are now available using lysine analogues which reduce germination and seedling growth of normal genotypes allowing selective recovery of "lysine overproducing" genotypes.

Objective 2 - Identify the chemical nature and composition of the protein fractions of selected high lysine sorghum genotypes and correlate the grain fraction composition with nutritional quality.

a. The Contractor shall develop methods to fractionate tannins for qualitative differences which might allow identification of sorghum lines in the collection with the desirable characteristics of tannin, including bird resistance, which do not interfere with the biological value of the grain (i.e., Van Soest method).

b. The Contractor shall make a comparison of ninhydrin and dye binding methods for screening seed genotypes from the world collection and from chemical mutagen-treated seeds. The ninhydrin and dye binding methods will be compared to determine whether both methods of screening should be used. The effect of tannins on both tests will be determined.

c. The Landry-Moureaux method of fractionation will be used to study mutant P721 and mutant 51016. The amino acid composition of each fraction shall be determined.

d. Acrylamide disc gel electrophoretic patterns will be obtained on the five protein fractions to explain variations in lysine levels in normal and high lysine genotypes. Any unique proteins found only in high lysine sorghums shall be isolated and characterized.

e. The high tannin sorghum BR64 shall be dehulled in a Palyi mill and the fractions obtained subjected to amino acid analysis, protein fractionation, and biological evaluation.

f. Protein efficiency ratios (PER), feed efficiency ratios (FER), and the new biological assay, relative protein value (RPV) shall be determined on selected normal and high lysine sorghums (both high and low tannin types) in the weanling rat. Dehulled sorghums will also be evaluated.

g. The Contractor shall isolate the polyphenolic compounds in high and low tannin sorghums by thin layer chromatography and attempt to identify the individual polyphenols. If any are unique to high tannin sorghums, a method of measurement can be devised which will be superior to the present catechin method.

h. Sorghum proteins from world collection lines shall be fractionated and lysine determinations of fraction V (glutelins) will be run to evaluate the extent of genetic variation available for selection. The glutelin fraction in sorghum is lower in lysine than that in maize. If extensive variation is found techniques shall be developed for rapid screening of

the glutelin lysine content of sorghum lines and mutants.

Objective 3 - Determine the nutritional value of sorghum varieties differing in amino acid composition, carbohydrate composition, and/or tannin content for the monogastric animal. Most of the research will be conducted with rats as the experimental animal.

a. Compare the nutritive value of low and high tannin sorghum grains from which the tannin containing testa layer has been removed by physical and chemical means.

b. Study the effect of tannin content of sorghum grains on protein, carbohydrate, and fat digestion.

c. Investigate the mechanism by which methionine alleviates the detrimental effects of tannin in the chick.

d. Evaluate the biological value of the high lysine mutant P721 opaque with P721 normal in chick and rat experiments. Also compare the heterozygous P721 opaque with corresponding homozygous grain.

e. Determine the biological value of any new sorghum lines which show favorable amino acid patterns by chemical analyses.

f. Compare high lysine sorghum lines with other cereal grains, such as corn, wheat, barley, triticale, and rice for biological value.

g. Determine the biological value of the most promising sorghums as human food.

h. Conduct long-term feeding studies with rats to ascertain the long range nutritional effects of selected improved sorghums.

Objective 4 - Determine the mechanism of inheritance of sorghum mutants and lines with improved nutritional quality and develop breeding methods and material, including populations with improved biological quality, for utilization in developing countries.

a. The Contractor shall continue development of high protein and high lysine male sterile (ms_3) random mating populations at Lafayette, Puerto Rico and other cooperative locations. The following populations are under development:

- A random mating population is being developed for tropical locations using high lysine germplasm in combination with Doggett's elite germplasm from Uganda.
- A random mating high lysine population containing the hl gene in combination with yellow and white endosperm germplasm is being developed for both temperate locations.
- A random mating high lysine population containing the hl gene and incorporating disease and insect resistant germplasm from Texas is being developed.

b. Evaluate S_1 progeny from the populations under item "a" (above) for protein, lysine, tannin, yield, and disease resistance at Lafayette, ICRISAT, ALAD, CIMMYT, as well as Puerto Rico, Brazil, Ethiopia and other national programs.

c. Initiate high lysine populations for temperate and tropical locations using the newly discovered high lysine gene (P721) as the source of protein quality. Approximately 500 crosses were made at Lafayette in 1974 to initiate the development of these populations.

d. Produce double mutant combinations between hl germplasm and mutant P721 to determine if there are additive effects on lysine content.

BUDGET FORECAST FOR 1976-77

Salaries	$141,440
Fringe Benefits	13,231
Overhead	96,886
Travel	11,200
Other Direct	4,400
Equipment & Supplies	28,541
Subcontracts	22,400
Consultants	1,000
TOTAL	$319,098

Purdue agronomist wins prestigious award

Dr. John D. Axtell, Purdue University agronomist, has received the 1976 Alexander von Humboldt Foundation award for "the most significant contribution to American agriculture in the past one to three years."

The award, a $10,000 cash gift, was presented this past week in special ceremonies in the Krannert Auditorium at Purdue by Karl-Hugo Schlunk of the Alexander von Humboldt Foundation and Dr. Lore Toepfer of Hamburg, Germany.

Axtell was honored for his contributions and leadership in improving the nutritive quality of sorghum. He is project leader for an interdisciplinary Agency for International Development (AID)-supported effort to improve the cereal. His research has led to two major breakthroughs in improving sorghum quality, both of which have impact worldwide on human and animal nutrition.

A MAJOR FINDING by Axtell and his associates was the discovery and identification of factors responsible for the reduced protein availability and digestibility in grain sorghum. They found that tannins in the colored testa layer under the seed coat greatly reduced the availability of proteins in digestion.

This knowledge permits simple genetic selection procedures for low tannin content, thereby increasing protein availability. This discovery has also led to research on other foods containing natural tannins.

The second major breakthrough was a high lysine mutant which, with low tannin, provides sorghum with a balanced amino acid content parallel to that of Purdue-discovered opaque-2 high lysine corn.

THESE TWO breakthroughs may be readily applied to improving the nutrition and well-being of the 300 million people in the world who depend on sorghum as a major protein and carbohydrate source in their diets.

Sorghum is the fourth most important cereal crop in the world, surpassed only by rice, wheat and corn. It will grow in the arid, stony soils of those areas where wheat and corn cannot be produced.

The award was made to Axtell by the Alexander von Humboldt Foundation, which is both a German and U.S. based organization funded by the Alfred Toepfer Co., a German trading firm. The foundation is named for the famous German geographer and naturalist of the 19th century.

THE FOUNDATION also presented a $3,000 scholarship to Darrell Schulze, a graduate student at Texas A & M University, who plans to continue his studies in soil mineralogy at the Technical Institute of the University of Munich in Germany.

Axtell was lauded at the presentation as "playing an historically singular role in the resolution of a problem confronting all mankind."

Dr. R. L. Kohls, Purdue's dean of agriculture, paid tribute to Axtell and his associates with this statement:

"ALL OF US in agriculture are thrilled that the von Humboldt Award has come to Prof. Axtell. The work involved is an excellent example of the problem-solving capacity of a well-directed and adequately funded research team. It is an excellent example of federal-state cooperation. It also demonstrates the far-reaching international implications of agricultural problems that face us today."

Also on hand to pay tribute to Axtell were Curtis Farrar, assistant administrator for technical assistance for the Agency for International Development; Dr. F. L. Patterson, assistant head of the Purdue Agronomy Department ; Dr. Fred Andrews, Purdue vice president and W. W. Lebeck, president of the Chicago Board of Trade.

Axtell joined the Purdue staff in 1967. He is a native of Minnesota and earned his B.S. and M.S. degrees at the University of Minnesota and his PhD. degree at the University of Wisconsin.

DR. JOHN D. AXTELL
. . .wins $10,000 prize