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ANNUAL REPORT

ON

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

January 1, 1974 - March 31, 1975

REPORT NO. 11

prepared by:

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Contract csd/1175

Submitted to:

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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/csd-1175.

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 (as amended): July 1, 1966 to March 31, 1975.

4. Period covered by report: January 1, 1974 to March 31, 1975.

5. Total AID funding of contract to date: $1,707,390.00.

6. Total expenditures and obligations through previous contract year: $1,707,388.19

7. Total expenditures and obligations for current contract year: $283,047.89

8. Estimated expenditures for the next contract year: (4-1-75 to 3-31-76): $299,978.00.

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 three 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 and utilization work.

D. ACCOMPLISHMENTS TO DATE.

We have been studying two major components of nutritional quality in grain sorghum. First, protein availability is apparently limited in some sorghum genotypes by the presence of unidentified polyphenolic compounds located primarily in the testa layer of the grain. These pigmented compounds have not been well characterized chemically and are referred to generically as "tannins". Second, the protein quality of sorghum is limited by the low lysine content of the grain which reflects the high prolamine content of the endosperm. If one looks at the essential amino acid composition of sorghum grain, in comparison with monogastric nutritional requirements, it is obvious that lysine is deficient and that there is great excess of leucine. The methionine content of sorghum is low, but considering the cystine content of 1.5%, the overall sulfur amino acid content approaches the weanling rat requirement. The tryptophan content of sorghum seems to be adequate in contrast to the low tryptophan content in normal maize.

Tannins.

Based on the above information one would predict that the biological value of any particular variety of sorghum grain would be directly proportional to its lysine content. This, in fact, is true for low tannin sorghum genotypes. The data in Figure 1, however, illustrate that lysine is not the first limiting component of biological value for a group of high tannin sorghum lines from the world collection. There is an important interaction between tannin content

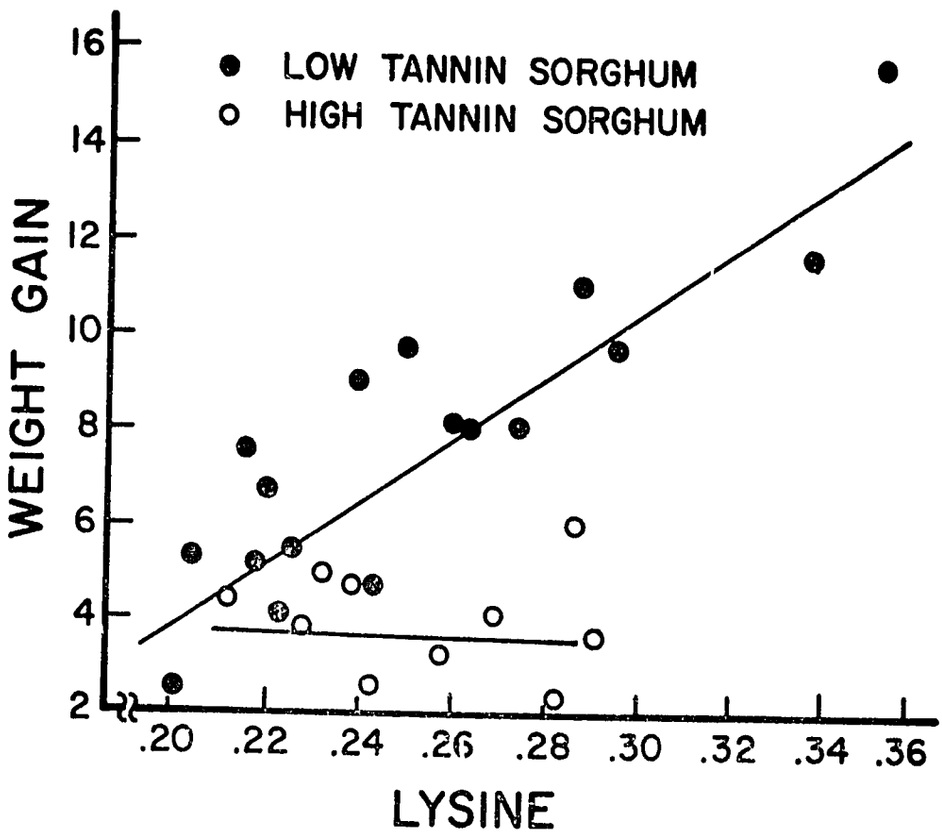

Figure 1. Relationship of biological value to lysine concentration (g/100 g sample) in high and low tannin sorghum lines from the world collection in a 14-day weanling rat feeding experiment.

and protein quality in sorghum which is not found in any of the other major cereals. Cummings and Axtell demonstrated this experimentally by feeding whole seed and dehulled high tannin sorghum grain IS 8260 as shown in Figure 2. Rat growth is poor for the whole grain IS 8260 with and without supplemental lysine. In contrast, dehulling IS 8260 grain improves biological value substantially, and also allows a significant rat growth response to the addition of supplemental lysine. A similar technique was used in this study to determine the quantitative effects of tannins on biological value. Using varying proportions of high tannin sorghum grain mixed with its dehulled low tannin counterpart we were able to provide diets containing a range in tannin content which were essentially isogenic comparisons. Data from this study is shown in Figure 3. The biological value as measured by rat weight gain is significantly reduced by increasing levels of tannin in the diet. We can conclude from this data that tannins reduce the biological value of grain sorghum for monogastric animals. The cause of this reduction is not completely understood. Our present hypothesis is that the seed proteins become complexed or bound with the tannins of the whole grain, and that the complexed proteins are substantially less available for utilization by monogastric animals. It is very important to recognize this tannin interaction with protein quality when assessing the biological value of any grain sorghum variety, since failure to consider the biological effects of tannins has led to substantial confusion in past studies of sorghum nutritional quality.

High Lysine Sorghum-Ethiopia.

In 1972 we initiated two approaches which we hoped would lead to the identification of sorghum lines with increased lysine content. The first of these approaches involved cross-sectioning of approximately 9,000 lines of sorghum from the world collection, and screening these sectioned kernels for

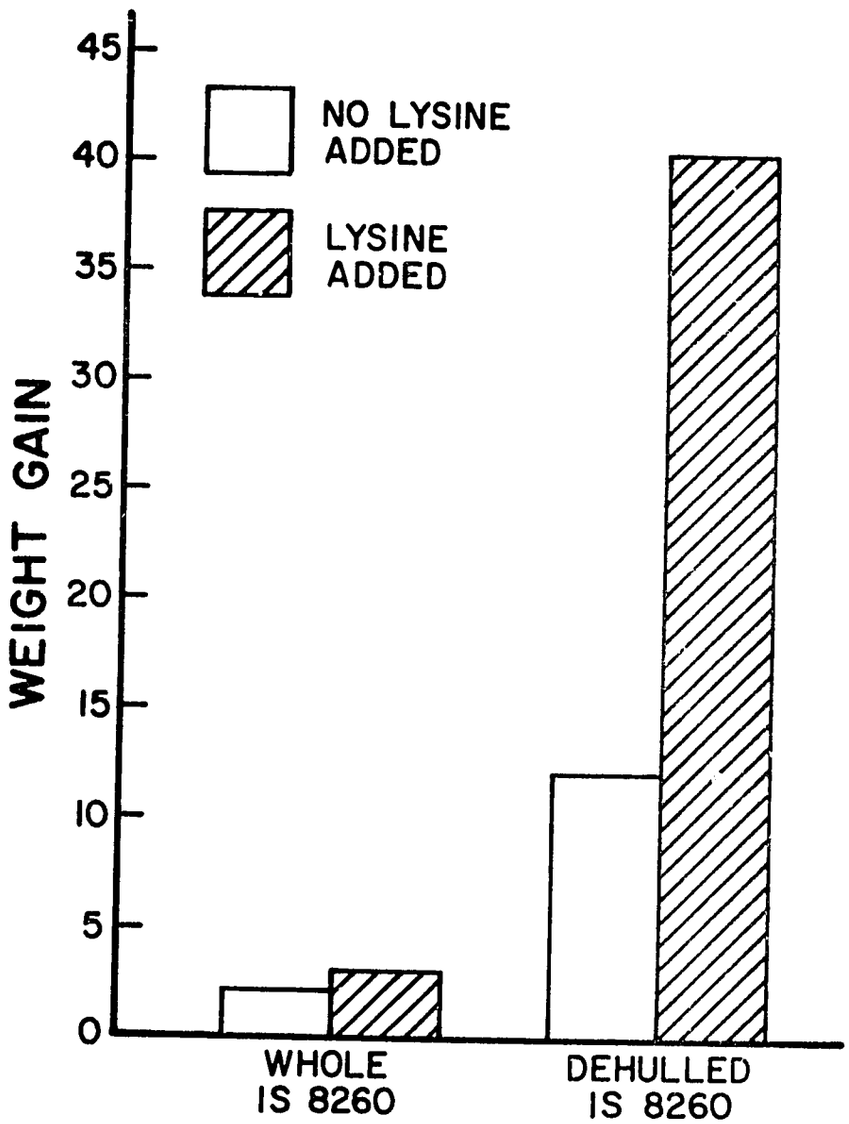

Figure 2. Biological value of whole and dehulled grain of high lysine sorghum line IS 8260 when fed to weanling rats with and without lysine supplementation in a 14-day feeding experiment.

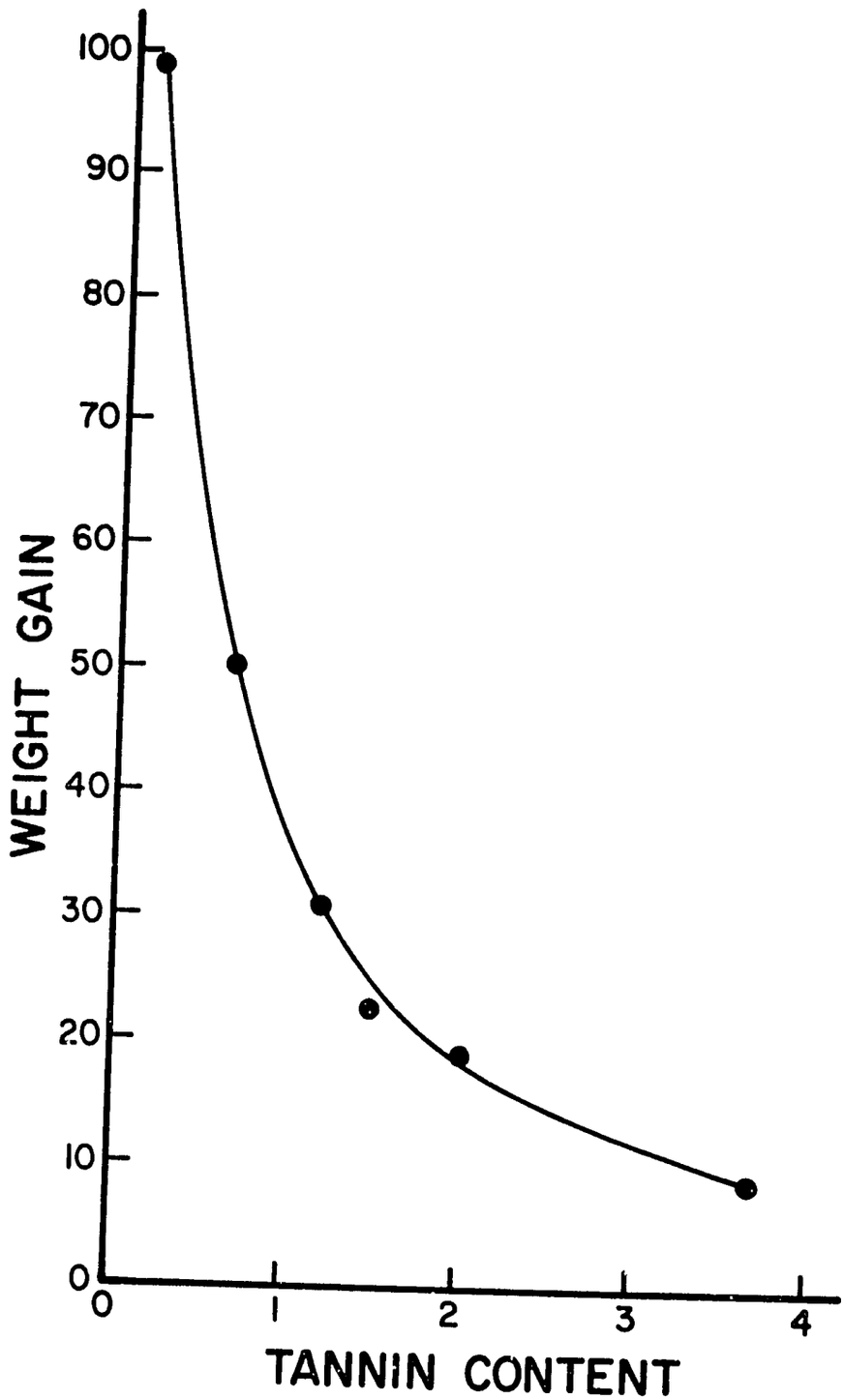

Figure 3. Effect of tannin content (expressed as catechin equivalents) on the biological value of sorghum grain in a 28-day weanling rat feeding experiment.

floury endosperm lines. Using this procedure, we identified 62 floury endosperm lines and evaluated each for lysine and protein content. Most of these floury endosperm lines were normal for lysine content in relation to their protein content. There were two floury endosperm lines, however, which had a high lysine content at relatively high levels of protein. IS 11167 and IS 11758 were both collected from Wollo Province in Ethiopia during the 1960's. The protein and lysine content of these two lines was subsequently verified in Puerto Rico, and the average of these determinations is shown in Table 1 in comparison with the average for several hundred normal sorghum lines from the world collection. The significant point is that lysine content when expressed as percent of sample is approximately double that of normal sorghum.

Table 1. Protein and lysine concentration of whole grain samples of high lysine and normal sorghum lines.

Protein Composition	High Lysine Lines		
	IS 11167	IS 11758	Normal
Protein (%)	15.70	17.20	12.70
Lysine (% of protein)	3.33	3.13	2.05
Lysine (% of sample)	0.52	0.54	0.26

The reason for this increase in lysine can be understood by looking at the distribution of endosperm proteins by solubility fractionation using the Landry Moureaux procedure. The distribution of protein in the five solubility fractions for normal sorghum is shown in Table 2, along with the lysine content of each of the five fractions. Over 50% of the endosperm protein in sorghum is present in Fractions II and III, which are both lysine-poor fractions. Fractions II and III are generally referred to as the prolamine fractions. Note that

Table 2. Protein and lysine distribution in normal sorghum endosperm.

Solubility Fraction*	Protein (%)	Lysine (% of protein)
I	7.3	5.1
II	16.8	0.3
III	32.4	0.3
IV	7.8	1.2
V	25.3	3.6

*Landry-Moureaux procedure.

Fractions I and V are relatively high in lysine. The nitrogen distribution in normal and high lysine sorghum endosperm tissue using the same procedure has been obtained by Jambunathan (Table 3, unpublished data). Data from

Table 3. Protein distribution in normal and Ethiopian high lysine sorghum endosperm.

	Protein (%)	
Solubility fraction*	Normal	High lysine (hl)
I	6.33	13.58
II	19.16	16.54
III	36.26	23.02
IV	6.65	4.00
V	25.60	33.86

*Landry-Moureaux procedure.

determinations on five high lysine lines collected from farmers' fields in Ethiopia were averaged and compared to control data from a normal variety grown in the same environment. There is a substantial reduction in the amount of protein in fractions II and III among the high lysine lines, accompanied by an increase in the proportion of protein in the lysine-rich fractions I and V. The reduction in prolamine from approximately 55% to 39% largely accounts for the increased lysine content of the Ethiopian lines. The high lysine content

of these Ethiopian lines is controlled by a single gene which has been designated hl. The biological value of the Ethiopian high lysine sorghum grain in comparison with normal has been reported by Singh and Axtell. Data obtained by feeding high lysine and normal samples at isonitrogenous levels (10% protein) to weanling rats in a 28-day trial demonstrated that rats fed the high lysine ration increased in weight approximately two to three times more than rats fed the normal control ration.

The kernel phenotype of the Ethiopian high lysine varieties is partially dented. This suggested the possibility that there may be a block in the biosynthesis of starch from water soluble polysaccharides similar to that found in shrunken, sugary, or brittle endosperm mutants in maize. The data in Table 4 shows the carbohydrate composition of both high lysine lines and a normal control. There is no change in the quantity of reducing sugars and only a slight increase in the amount of sucrose in the high lysine lines. Water soluble polysaccarides content is similar to that of normal, and there is only a two to three percent reduction in starch which can be accounted for by the slight elevation in the levels of sucrose. We concluded, therefore, that there is no disruption in starch synthesis from water soluble polysaccharides in hl genotypes, and that it may be possible to identify plump seeded high lysine kernels in an appropriate genetic background.

We travelled to Wollo Province, Ethiopia, in November of 1973 to see if we could find these same high lysine varieties under cultivation, and if possible to find out how the people were using them. In fact, we first found them growing within one or two kilometers of the site where they were originally collected, thanks to some excellent records of Dr. Jack Harlan. Seeds of the high lysine varieties were collected in cooperation with Dr. Brhane Gebrekidan, who is in charge of the sorghum breeding program at Alemaya, and

Table 4. Carbohydrate composition of whole kernel samples of Ethiopian high lysine and normal sorghum lines.

Carbohydrate Composition	High lysine lines IS 11167	IS 11758	Normal
Reducing sugars	0.38	0.32	0.34
Sucrose	3.08	2.61	1.03
Total sugars	3.46	2.93	1.34
Water soluble polysaccharides	0.91	1.01	1.11
Starch (% of sample)	58.90	57.80	60.80
Total carbohydrates	63.27	61.74	63.25

Mr. Gebisa Ejeta, a graduate assistant from Alemaya, who is now studying at Purdue. The high lysine types were always found growing in mixtures with normal sorghum varieties--a standard practice for most of the sorghum cultivated in Ethiopia. The local farmers could recognize and identify the high lysine varieties and reported to us that it was used primarily for roasting in the late dough stage. They also recognized that the yield of this variety was lower than that of their normal sorghum varieties, but they included it in the mixture because they liked the taste of the roasted grain.

High Lysine Sorghum - Induced Mutation.

The second approach initiated in 1972 was the use of chemical mutagen treatments, specifically diethyl sulfate, to try to induce a mutant which would increase the lysine content of the sorghum grain. D. P. Mohan screened 23,000 M_3 heads during his Ph.D. thesis research at Purdue for the occurrence of opaque kernel mutants. All heads from treated seeds were bagged each generation to prevent outcrossing. Seeds from each M_2 head were placed over a light box and examined for segregating opaque kernels. Mohan identified approximately 475 heads segregating for opaque kernels, and both classes of seed from each segregating head were analyzed for protein and lysine content. The percent increase in lysine of these 475 putative

opaque mutants is shown in Table 5. Most of the opaque mutants were normal in lysine content, but there were 33 with at least a 50% increase in lysine. The mutant from this group that appears to have the most promise at this time is P721. The average protein and lysine content of P721 opaque seed and normal sib seed is shown in Table 6. There is an appoximately 60% increase in lysine in the P721 mutant grain.

Table 5. Distribution of lysine increases (% of sample) of 475 putative opaque endosperm sorghum mutants derived from diethyl sulfate treatments.

Percent increase in lysine	Number of putative opaque mutants
0-9	135
10-19	114
20-29	85
30-39	53
40-49	25
50	33

Table 6. Protein and lysine composition of high lysine opaque mutant P721 and normal sib grain.

	Protein (%)	Lysine (% of protein)	Lysine (% of sample)
P721 opaque	13.9	3.09	0.432
Normal sib	12.9	2.09	0.270

The inheritance of this opaque mutant is not yet clearly understood, but it appears to be controlled by a single gene which expresses partial dominance in the triploid sorghum endosperm. In some cases, F_2 segregation ratios fit a 1:1 ratio, but in others there is a deviation in favor of more opaque and fewer vitreous kernels. This ratio would be expected if two or three doses of

the opaque gene produce opaque kernel phenotypes, whereas one dose of the opaque gene in the triploid endosperm is recessive to normal vitreous expression. We expect that this ratio will vary with genetic background.

A comparison of the biological value of the Ethiopian high lysine sorghum with the newly recovered mutant P721 is presented in Table 7. This data was obtained from two different feeding trials using a standard ANRC casein diet as a check which allows comparison between trials. RS610 was used as a normal sorghum control in the IS 11758 trial, whereas normal sib seed of mutant P721 was used as a normal control in the P721 evaluation. All rations were isonitrogenous (10% protein) and were fed to weanling rats for 28 days. Feed efficiency ratios and protein efficiency ratios of both high lysine mutants appear to be similar, and we conclude from this preliminary experiment that the protein quality of both sources of high lysine sorghum germplasm is essentially the same. That is, both sources of high lysine germplasm give an approximately two-fold increase in protein quality in comparison with the casein control.

Table 7. Biological value of isonitrogenous (10% protein) diets of Ethiopian (IS 11758) and P721 high lysine sorghums and their respective normal checks, RS 610 and normal sib grain in a 28-day weanling rat feeding experiment.

	Weight gain (g) (28 days)	FER	PER	% of casein
IS 11758	40	5.8	1.7	58
RS 610	16	10.5	0.9	32
P721	33	5.7	1.8	64
Normal sib	11	10.6	0.9	32

Since mutant P721 was recovered in a photoperiod insensitive, three-dwarf line with relatively broad agronomic adaptability, this mutant may be most readily useful in temperate sorghum growing regions of the world. The increased protein content of the Ethiopian high lysine varieties, on the other hand, may provide useful sorghum germplasm for use as a protein supplement or weanling food for young children which is needed in many parts of Africa and Asia.

Future Research.

The prolamine contents of the major cereals fall into three rather distinct groups, as shown in Figure 4. Since the protein quality of cereals is inversely related to their prolamine content, these cereal groups increase in protein quality as they decrease in prolamine content. (This is an over-simplification which the nutritionists would justifiably dispute. There are numerous other parameters affecting nutritional quality in cereals. Nevertheless, the improvement in biological value associated with "high lysine" mutations in maize, barley, and sorghum can be largely attributed to a reduction in the proportion of prolamine in the endosperm, together with concomitant enrichment in the proportions of albumins, globulins, and glutelins.) This figure also shows the changes in prolamine content associated with high lysine mutants in three of these cereals. These changes appear to occur in two steps from higher to lower prolamine content. Dr. Oliver Nelson has suggested that this may be coincidence or it may mean that the regulation of prolamine synthesis is controlled by two major genes in these cereals. If the latter interpretation proves to be correct, it should be possible to find a "second-step" mutation in sorghum (or corn) which will reduce the prolamine content to approximately 10%, with further concomitant increases in protein quality. This would provide us with a cereal grain which combines the photosynthetic efficiency and stress

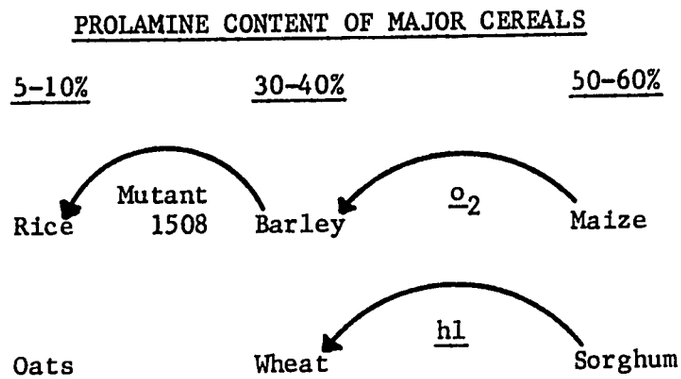

Figure 4. Effect of high lysine gene mutations in maize (o_2), sorghum (hl) and barley (1508) on prolamine content.

tolerance of sorghum with the protein and nutritional quality of oats or rice.

We can speculate still further into the future in our search for a more "perfect cereal" and ask the following question. Will it be easier to breed for increased photosynthetic efficiency in rice or oats, or to take the alternative approach and breed for nutritional quality in sorghum and corn which already have the genetic machinery for a more efficiency photosynthetic pathway? The answer will depend on which biochemical process is easiest to manipulate genetically, which in turn can only be decided when we understand the genetic control of these important biochemical pathways in cereal grain.

E. DISSEMINATION AND UTILIZATION OF RESEARCH RESULTS

1. Dissemination of Results.

a. Research reports.

Approximately one thousand copies of the 1973 research report were printed with copies sent to over 800 cooperators in 109 states and countries. The computerized mailing list has been sent to India, Nebraska, Lebanon, Puerto Rico and Pakistan, as requested for their use in contacting fellow researchers.

The Grain Sorghum International Protein Yield and Quality Trial, Report No. 1, Station Bulletin No. 52 was distributed to all cooperators for their use in identifying broadly adapted high yielding varieties and comparing their observations to other locations for adaptability and potential usage of selected germplasm.

b. Breeder samples of high lysine genotypes.

In addition to the requests of IS 11758 and IS 11167 filled in 1973, breeder samples of seed were sent to 46 additional cooperators representing 24 countries and 11 states who requested this high lysine breeding material this year. During the year these lines have been distributed to workers in the following countries and states: Brazil (5), Uruguay (1), Colombia (1), Peru (1), Venezuela (1), Mexico (2), Honduras (1), Haiti (2), Virgin Islands (1), Australia (1), Russia (1), Italy (1), Romania (1), Germany (1), Hungary (1), France (2), Lebanon (1), India (1), Korea (1), Indonesia (1), Yeman (1), Viet Nam (1), Dahomey (1), Niger (1), Uganda (1), Nigeria (3), Zaire (1), Sri Lanka (2), Tanzania (1), Hawaii (1), Puerto Rico (1), Kansas (4), Texas (5), Illinois (1), Nebraska (3),

Pennsylvania (1), Mississippi (1), Louisiana (1), Arkansas (1), Oklahoma (1), Tennessee (1), Maryland (1), Minnesota (1), and Georgia (1).

The release in late 1974 of the chemically induced opaque mutant P-721 selected from a partially converted line from the Texas Conversion Program resulted in requests for breeder samples from the following: Argentina (1), Venezuela (1), Mexico (1), Viet Nam (1), Yeman (1), Lebanon (1), Dahomey (1), India (1), Maryland (1), New Mexico (1), Illinois (3), Texas (30), Iowa (2), Minnesota (3), Kansas (2), South Carolina (1), California (2), Colorado (1), Arizona (2), Michigan (2), and Georgia (1). This represents nine workers from nine countries, and 57 workers from 14 states.

c. Seminars and Papers Presented.

Axtell, J. D. "High Lysine Sorghum" Funk's International, Bloomington, Illinois. February 4-5, 1974.

Oswalt, D. L. "Improved Protein Quality in Cereal Crops: Present Utilization and Future Possibilities" First World Population Society Meetings, Washington D. C. February 5-8, 1974.

Axtell, J. D. Attended Workshop on "Genetic Improvement of Seed Proteins" Washington, D. C. Sponsored by National Academy of Science. March 17-22, 1974.

Axtell, J. D. "Improved Nutritional Quality of the World's Food Grains" Symposium for Modernization and Development. Purdue University, W. Lafayette, Indiana. March 27, 1974.

Oswalt, D. L. "Crop Development and Nutrition, The Case for Sorghum" Symposium for Modernization and Development. Purdue University, W. Lafayette, Indiana. March 27, 1974.

Axtell, J. D. "Sorghum Improvement at Purdue" Sorghum Improvement Conference of North America. Lincoln, Nebraska. September 4-6, 1974.

Oswalt, D. L. "Prospects in Dryland Cereals, Needed Work and Opportunities" Seminar on Dryland Zone of Central West Africa. Washington, D. C. October 3, 1974.

Featherston, W. R. "Poultry Nutrition Studies". Indiana Poultry Industry Conference. Purdue University, W. Lafayette, Indiana. April 1974.

Axtell, J. D. "Genetic Improvement of the Quality and Quantity of Food Grains". Symposium on World Food Problems and Issues. Purdue University, W. Lafayette Indiana. November 1974.

Oswalt, D. L. "Identifying Grain Sorghum Varieties with a Testa and Low Concentrations of Tannin". American Society of Agronomy meetings, Chicago, Illinois. November 1974.

Featherston, W. R. "Studies to Improve the Nutritional Value of Sorghum Grain". Purdue University TV series entitled Agriscope. December 1974.

Axtell, J. D., D. P. Mohan and Donn Cummings. "Genetic Improvement of Biological Efficiency and Protein Quality in Sorghum". American Seed Trade Association Meetings, Chicago, Illinois. December 1974.

Mohan, D. P. and J. D. Axtell. "Protein Quality Improvement in Grain Sorghum". International Sorghum Workshop, Mayaguez, Puerto Rico. January 1975.

Porter, K. S., J. D. Axtell, V. L. Lechtenberg, and V. F. Colenbrander. "The Brown Midrib Gene (brm) in Sorghum and its Affect on Forage Quality". International Sorghum Workshop, Mayaguez, Puerto Rico. January 1975.

Oswalt, D. L. "Estimating the Biological Effects of Tannins in Grain Sorghum". International Sorghum Workshop, Mayaguez, Puerto Rico. January 1975.

Oswalt, D. L. "Management Practices and Factors Limiting Sorghum Yields in Low Production Situations". International Sorghum Workshop, Mayaguez, Puerto Rico. January 1975.

Featherston, W. R. and J. C. Rogler. "Studies on the Nutritional Quality of Sorghum Grain." International Sorghum Workshop, Mayaguez, Puerto Rico. January 1975.

Axtell, J. D. "Sorghum Nutritive Value - Present and Future". DeKalb AgResearch Biennial Research Conference. Sea Island, Georgia. March 1975.

Axtell, J. D. "Protein Improvement - World-Wide Application". Ninth Biennial Grain Sorghum Research and Utilization Conference. Lubbock, Texas. March 1975.

d. Selected Germplasm Utilization.

The Second International Protein Yield Trials (Study 398) initiated in 1973 are identifying additional lines having a wide adaption. Cooperators have identified lines which they are releasing for increase or placing in their breeding programs. The Third International Protein Yield Trials (Study 498) were selected and sent upon request to:

	(no. of sets sent:)				
Asia	Afghanistan	1	Africa	Egypt	2
	Bangladesh	5		Ivory Coast	2
	India	4		Niger	3
	Pakistan	4		Nigeria	2
	Philippines	1		Sudan	1
	Sumatra	1		Tanzania	2
	Thailand	2		Chad	1
	Indonesia	2		Dahomey	1
	Viet Nam	2		Liberia	4
Latin America	Argentina	4	United States	Hawaii	2
	Bahamas	1		Nebraska	1
	Brazil	19			
	Honduras	1			

Results from the 398 study distributed in 1973 are now being returned and data to date indicate a wide range of adaptability and acceptability in line response as was indicated by the different germplasm selections in the 298 study. (Purdue University Agricultural Experiment Station Bulletin No. 52, 1974).

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

Contact with sorghum workers continues to increase as correspondence was maintained with workers as follows:

	No. of countries or states	No. of workers
Africa	20	69
Asia	20	116
Australia (area)	3	19
Europe	11	23
N. America	36	103
S. America	19	137
	109	467

Numerous selections continue to be made from requested germplasm which was provided by this project. Park/Korea has helped release IS 8766 for farmer use. Whiteman/Botswana indicates that IS 3977, 932354, IS 0628, 954030, IS 0508, and 954015 have been selected as elite lines for use there;

Nider/Argentina selected IS 3441 from the 398 yield trials; Westenberg/ Indonesia reported that 954243 has been planted widely and was the best in the lowlands of all varieties tested, while 954149 was the best for the highlands. Moore/Bangladesh selected 398001, 398006, 398009, and 398109 for increase along with 398014, 398018, and 398023. In Thailand IS 8719 has been high yielding with shootfly tolerance identified in IS 5604 (best) and IS 3796, IS 3922, IS 0084 and IS 0511. IS 3796, IS 3922, IS 0084, and IS 0511 were selected as good yellow varieties and bird resistant selections of IS 9181 and IS 8719 were made in Thailand and some additional nine other lines were added to their breeding program. Liberian workers identified 498026 and 498027 as rapid maturing varieties suitable for multiple cropping and 498001, 498006, 498008, 498021, 498022, 498023, 498024 and 498028 as very high yielding varieties. Disease and insect resistance have been identified by the Texas and Georgia Experiment Station evaluation nurseries.

Special requests for specific purposes and types of seed were answered from: Johnson/Mexico, Ellis/Liberia, Wannemacher/Germany, Doggett/ICRISAT-India, Arogun/Nigeria, Sandlin, Rosenow and Miller/Texas, Rao/India, Sanchez/ Brazil, Hoegemeyer and Eastin/Nebraska, Hadley/Illinois, Herrenthey/Bangladesh, Whipple/Oregon, Kopf/Viet Nam, Gourley/Mississippi, Cummins/Georgia, Unruh/ Paraguay, Riley/Ethiopia, Pacagnini/Argentina, House/Lebanon, Faris and Schaffert/Brazil, Tarumoto/Japan, and Denis/Senegal. This accounts for 14 countries and six states and 26 individuals.

3. Plans to Use Research Data More Efficiently.

A Latin American Sorghum Workshop in cooperation with the workers from

Texas, Nebraska, and Puerto Rico was held in Puerto Rico in January 1975. An additional workshop is being planned for Africa to identify problems related to the production of improved protein quality sorghum and to facilitate the exchange of information among sorghum research personnel in that region.

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 continued with international centers (ICRISAT, ALAD, and CIMMYT) and with the sorghum research projects in Puerto Rico, Texas, and Nebraska.

4. Involvement of LDC or US Personnel and Institutions.

Students from Ethiopia (Gebisa Ejeta), Lebanon (Vartan Guiragossian on a Rockefeller Fellowship), and the United States (Paul Christensen with Peace Corps experience in Ethiopia, John Clark with experience in Viet Nam, Steve Van Scoyoc and K. S. Porter)started graduate training during the year with the project personnel.

Extensive correspondence related to data and germplasm distribution related to the hl gene (IS 11758 and IS 11167 released in 1972) and the P-721 mutant released in late 1974 resulted in project personnel having increased contact with commercial, state, and related sorghum research projects. The request for data and germplasm by ICRISAT, ALAD, and CIMMYT from this project continues to broaden the potential adaptation of the high lysine germplasm for eventual acceptance of improved quality varieties in LDC's. Contacts with workers in the following countries have

been carried on with one or more rounds of correspondence where data and/or germplasm have been furnished when requested: Argentina (2), Austria (1), Australia (1), Bahamas (1), Bangladesh (1), Botswana (1), Brazil (6), Canada (3), Chile (1), Colombia (3), Cameroun (3), Denmark (1), Ethiopia (4), France (3), Germany (2), Guatemala (1), Haiti (1), Hungary (2), India (7), Indonesia (4), Italy (1), Kenya (2), Liberia (1), Malaysia (1), Mexico (5), Netherlands (1), Nicaragua (2), Niger (3), Nigeria (7), Paraguay (1), Peru (1), Phillippines (1), Puerto Rico (4), Romania (1), Russia (1), Senegal (2), Sri Lanka (1), Tanzania (1), Thailand (2), Togo (1), Uruguay (1), Venezuela (2), Viet Nam (2), Virgin Islands (1), Yeman (1), Arizona (2), California (4), Colorado (1), Connecticut (2), Delaware (1), Florida (2), Georgia (5), Hawaii (1), Illinois (6), Iowa (1), Kansas (5), Kentucky (1), Louisiana (1), Michigan (2), Minnesota (1), Mississippi (1), Nebraska (6), New York (3), North Carolina (3), Ohio (1), Oklahoma (1), Oregon (1), Pennsylvania (1), Tennessee (1), Texas (12), Virginia (1), and Wisconsin (2). This represents overseas correspondence with 47 countries (141 letters) and 96 cooperators and 21 states (97 letters) with 68 different workers.

Visitors to the project and its personnel continue to provide an opportunity to stimulate research and improved germplasm utilization. Those visiting were: Salamini, Lorenzoni, Maggione/Rome, Italy; Rhoad/AID-Washington; Boulos/Egypt; Hulse/Canada; Lawrence/ICRISAT-India; Lucas, Callis (representing AFRICARE; Rabson/Austria; Hadley/Illinois; Ingversen/Denmark; Hudson, Early/ Indiana, Naudt/Venezuela; Abdoulaye, Assa, Oulai, Bosso, Zahabi, Benjamin, Hyacinthe, Marchanna/Ivory Coast; Ahoua, Diarra/Washington (Ivory Coast Embassy); Botha/S.Africa; Satzapal/UNDP (New York); Ferrer/Phillippines; Perkinson/Illinois; Shelten/Puerto Rico; Wray/Scotland; Saibro/Brazil;

Riley, Worede/Ethiopia; Marcano/Venezuela; Mumba/Zambia; Vieira/Brazil; McWhirter/Australia; Benors/France; House/ALAD-Lebanon; Lafantant/Haiti; Rosechlamb/Indiana; Berry/Texas; Baird, Holmes/AID-Washington; Imorou/Dahomey; Tarumoto/Japan; Zalnt/Hungary; Gul/Afghanistan; and Brewbaker/Hawaii. This represents 51 visitors from 29 countries or states.

F. STATEMENT OF EXPENDITURES AND OBLIGATIONS AND CONTRACTOR RESOURCES.

Category:	Total Adjusted Budget Amount 4/1/74 - 3/31/75	Expenditures 4/1/74 - 3/31/75
Salaries and Wages	$121,000	$121,483.87
Overhead	82,885	83,216.46
Salary Related Costs	10,610	10,168.20
Non-Expendable Equipment	5,650	5,392.25
Expendable Equipment and Supplies	19,017	18,154.68
Winter Nurseries	12,500	12,500.00
Travel and Subsistence	10,954	8,470.60
Computer Services, Publications, and Workshops	6,460	7,638.63
Subcontract Analyses	15,924	16,023.20
	285,000	283,047.89*

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* As of July 31, 1975 adjusted.

G. WORK PLAN AND BUDGET FORECAST FOR 1975-78.

Objective 1 - Identify and evaluate sorghum lines or mutants with improved protein quality and quantity 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 (1975-78).

- Tannin content will be estimated as catechin equivalents which has been a reliable assay that is consistently negatively correlated with rat response.

- The high tannin sorghum lines will be analyzed for lysine content using short column ion-exchange chromatography on the Beckman amino acid analyzer.

- The low tannin sorghum lines will be screened for lysine content using the dye binding technique and a newly developed ninhydrin method followed by rat feeding trials on the best entries.

b. Evalute M_3 progeny of high lysine opaque (P721) lines treated with chemical mutagens in 1974-75 for the identification of vitreous endosperm mutants (su_2 types) (1975-78).

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 (1976-78).

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

and biological evaluation (1975-76).

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 u. 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 (1975-78).

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 (1975-76).

c. The Landry-Moureaux method of fractionation will be used to study mutant P721 (1975-76) and mutant 51016 (1976-77). 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. P721 shall be studied in 1975-76 and mutant 51016 in 1976-77. 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 (1975-76).

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 (1975-78). Dehulled sorghums will also be evaluated (1975-76).

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 (1975-78).

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 and/or tannin 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 (1975-76).

b. Study the effect of tannin content of sorghum grains on protein, carbohydrate, and fat digestion (1975-76).

c. Investigate the mechanism by which methionine alleviates the detrimental effects of tannin in the chick (1975-76).

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 (1976-77).

e. Determine the biological value of any new sorghum lines which show favorable amino acid patterns by chemical analyses (1976-77).

f. Compare high lysine sorghum lines with other cereal grains such as corn, wheat, barley, triticale, and rice for biological value (1976-77).

g. Determine the feasibility of using a pilot organism such as *Tribolium confusm* to screen sorghum samples for biological value (1977-78).

h. Study the effects of storage and water extraction of high tannin sorghums on their nutritive value (1977-78).

i. As greater quantities of grains become available investigate the biological value of high tannin and high lysine sorghum for swine (1977-78).

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

k. 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 high 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 (1975-78). 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 and tropical 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 (1975-78).

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.

e. Evaluate the inheritance, chemical composition, and biological value of a recently identified line from Cameroon which has a high lysine content in a vitreous endosperm phenotype. (This is preliminary evidence and must be confirmed by progeny testing during the 1974-75 Puerto Rico grow-out).

f. Continue protein yield trials with 18-20 cooperators in the U.S. and overseas using a new set of 25 high lysine lines per year (study 598-798).

Additional Plans: Make available and distribute collected and developed information for improved management and high performance of sorghum, distribute collected germplasm and develop cooperative programs to test, screen and exchange breeding materials.

a. Breeding and evaluation material will be distributed to cooperators as follows:

- Selected diverse lines for breeding sources will be sent to cooperators requesting seed. Instructions for management, data collection and return of select grain samples useful for study will accompany the seed shipment. Seed of selected lines and all data will be returned for analyzing.

- Populations and segregating materials (one or two random mating high protein populations) as requested.

b. The Contractor shall print and distribute catalogs of suitable material to enable adequate utilization of data related to origin, composition, inheritance and nutritional quality of populations and collection items, if needed.

c. M.S. and Ph.D. degree programs with theses on the work related to this project shall be continued at about the level of three candidates at a time.

d. Non-degree programs shall be conducted (number determined largely by cooperators) with response by this project to the following:

- Continuation of visitors from many countries.
- Short term AID-supported individuals and groups.
- Longer term (six months to one year) visiting professors and others.
- Commercial company and other trainees.
- Special research problems by superior Purdue undergraduate students.
- Postdoctorates (two full-time and one quarter-time position funded by this project).

e. The Contractor shall initiate and develop plans for a workshop to be held in Africa as soon as feasible to be followed by one in Asia.

f. Initiation of opportunities to share findings and problems with coworkers shall be promoted by visits to the Puerto Rico, Texas, and Nebraska sorghum programs, overseas cooperators, attendance at relevant seminars and conferences, inviting coworkers to visit the project's nursery and laboratories, Purdue Agronomy Farm Field Day participation and continued cooperation with the Indiana Agricultural Extension Service.

Budget 1975-76:			
	line item 1	salary and wages	$ 134,865
	2	salary related costs	11,030
	3	indirect costs (68.5%)	92,383
	4	travel	11,000
	5	other direct costs	4,000
	6	permanent equipment, vehicles, materials and supplies	29,200
	7	subcontract services	16,300
	8	consultants	1,200
			299,978

H. APPENDICES

Bibliographic List and Short Abstracts of Research Reports for 1974 Representing Efforts to Disseminate the Results of the Research Project.

Armstrong, W. D., W. R. Featherston, and J. C. Rogler. 1974. Effects of bird resistant sorghum grain and various commercial tannins on chick performance. Poul. Sci. 53:2137-2142.

Armstrong, W. D., J. C. Rogler, and W. R. Featherston. 1974. In Vitro studies of the protein digestibility of sorghum grain. Poul. Sci.53:2224-2227.

Featherston, W. R., J. C. Rogler, J. D. Axtell and D. L. Oswalt. 1975. Nutritional value of high lysine sorghum grain for the chick. Poul. Sci. (In Press)

Featherston, W. R. and J. C. Rogler. Studies on the nutritional quality of sorghum grain. Proceedings of the International Sorghum Workshop, Mayaguez, Puerto Rico. (In Press)

Jambunathan, R., E. T. Mertz, and J. D. Axtell. 1975. Fractionation of soluble proteins of high lysine and normal sorghum grain. Cereal Chem. 52:119-111.

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

Mohan, D. P. and J. D. Axtell. 1974. Chemically induced high lysine mutants in sorghum bicolor (L.) Moench. Agron. Abstracts, p. 66.

Mohan, D. P. and J. D. Axtell. 1975. Protein quality improvement in grain sorghum. Proceedings of the International Sorghum Workshop, Mayaguez, Puerto Rico. (In Press)

Oswalt, D. L. and J. D. Axtell. 1974. Identifying grain sorghum varieties with a testa and low concentrations of tannins. Agron. Abstracts, p. 107.

Oswalt, D. L. 1975. Management practices and factors limiting sorghum yields in low production situations. Proceedings of the International Sorghum Workshop, Mayaguez, Puerto Rico. (In Press)

Oswalt, D. L. 1975. Estimating the biological effects of tannins in grain sorghum. Proceedings of the International Sorghum Workshop, Mayaguez, Puerto Rico. (In Press)

Oswalt, D. L. 1974. Improved protein quality in cereal crops: present utilization and future possibilities. Proceedings of the World Population Society meetings, Washington, D. C.

Porter, K. S., J. D. Axtell, V. L. Lechtenberg, and V. F. Colenbrander. 1975. The brown midrib gene (brm) in sorghum and its affect on forage quality Proceedings of the International Sorghum workshop, Mayaguez, Puerto Rico. (In Press)

ABSTRACT

EFFECTS OF BIRD RESISTANT SORGHUM GRAIN AND VARIOUS COMMERCIAL TANNINS ON CHICK PERFORMANCE. W. D. Armstrong, W. R. Featherston, and J. C. Rogler.

Studies were conducted to determine: (1) the effect of protein level on the performance of chicks fed bird resistant sorghum grains; (2) the influence of two levels of DL-methionine in alleviating the detrimental effects of bird resistant sorghum grains; (3) the effects of various sources of commercial tannic acid on chick performance.

Bird resistant sorghum (BR64) produced poorer chick performance than non-resistant sorghum (RS610) in sorghum-soybean meal diets containing 16 and 20% dietary protein. Addition of 0.15% DL-methionine to both sorghum grain diets resulted in significant improvements in chick performance, but the magnitude of the response was greater with the bird resistant than with the non-resistant sorghum grain diets. After methionine supplementation, the BR64 diets supported similar chick weight gains as the RS610 diets at both dietary protein levels; however, feed efficiency of chicks fed the BR64 diets remained poorer.

In additional studies, sorghum RS610 supported significantly better chick performance than either bird resistant sorghum BR64 or sorghum RS610 plus 1% isolated tannic acid. The addition of 0.15% DL-methionine to sorghum RS610 and BR64 diets resulted in significant improvements in chick weight gains with no further increase being noted when 0.30% supplemental DL-methionine was fed. Adding methionine to the RS610 plus tannic acid diet had little effect on chick weight gain. Supplemental methionine resulted in significant improvements in feed conversion with both the BR64 and RS610 plus tannic acid diets.

Addition of tannic acids of varying molecular weights to a non-resistant sorghum grain diet resulted in significant depressions in chick performance. The detrimental influence of the tannins did not appear to be closely associated with their molecular weights as all depressions in chick weight gain and feed conversion were roughly similar. The supplementation of 0.15% DL-methionine to diets containing each of the tannic acids had little effect on improving chick performance.

Poultry Science 53:2137-2142, 1974

ABSTRACT

IN VITRO STUDIES OF THE PROTEIN DIGESTIBILITY OF SORGHUM GRAIN.
W. D. Armstrong, J. C. Rogler, and W. R. Featherston.

Two non-bird resistant grain sorghums exhibited higher *in vitro* protein digestibility values than two bird resistant varieties. Extraction of tannins from a bird resistant variety resulted in increased *in vitro* protein digestibility as compared to the non-extracted grain, whereas the extraction process had little influence on the protein digestibility of a low tannin non-resistant grain sorghum.

Poultry Science 53:2224-2227, 1974

ABSTRACT

NUTRITIONAL VALUE OF HIGH LYSINE SORGHUM GRAIN FOR THE CHICK.
W. R. Featherson, 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 (IS 11758) with two low tannin commercial sorghum grains (RS671), (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 (In Press)

ABSTRACT

STUDIES ON THE NUTRITIONAL QUALITY OF SORGHUM GRAIN.
W. R. Featherston and J. C. Rogler

High tannin bird resistant sorghum grains have been shown to produce poorer growth and feed efficiency of chicks and rats as compared with the performance noted with low tannin non-resistant grains. Extraction of the tannins from bird resistant sorghum resulted in chick performance equal to that noted with chicks fed low tannin sorghum grain. Thus, the biological value of the various grains appears to be approximately equal once the detrimental effects of the tannins have been removed.

Digestibility studies with rats demonstrated significantly lower protein and caloric digestibility by rats fed a bird resistant grain as compared with rats fed the same grain with the tannins removed or a non-resistant sorghum grain.

The detrimental effects of the tannins in sorghum grain can be overcome by the supplementation of chick diets with methionine. Similar results have not been observed with rats. The manner in which methionine exerts its effect remains obscure. The addition of 1.0% polyvinylpyrrolidone to the diet has been shown to overcome the effects of both sorghum grain tannins and commercial tannic acid.

Proceedings of the International
Sorghum Workshop, Mayaguez, Puerto
Rico, January 1975 (In Press).

ABSTRACT

FRACTIONATION OF SOLUBLE PROTEINS OF HIGH LYSINE AND NORMAL SORGHUM GRAIN.
R. Jambunathan, E. T. Mertz, J. D. Axtell.

The discovery that opaque-2 and floury-2 mutant strains of maize had an improved protein quality has stimulated considerable research on the improvement of protein quality of other cereals. A mutant gene (hl), similar to opaque-2, was recently identified in sorghum by Singh and Axtell. After screening over 9000 lines in the world sorghum collection, Singh and Axtell were able to select two dented floury lines of Ethiopian origin, IS 11758 and IS 11167 which are high in lysine concentration at relatively high levels of protein.

When the opaque-2 gene is incorporated into normal maize, the prolamine (zein) is reduced and the glutelines, albumins and globulins increased, resulting in a larger amount of lysine in the whole kernel. In this communication we report the distribution of soluble protein fractions in high lysine sorghum kernels and compare them with the fractions in the normal sorghum with similar genetic background. Phenotypically the high lysine sorghums, IS 11167 and IS 11758, can be easily distinguished from normal sorghum by their floury and dented endosperms. By normal sorghum, we mean those which are similar to varieties and hybrids in commercial production having rounded kernels with some vitreous endosperm and low lysine content. A cross was made between high lysine sorghum (IS 11758) and the normal variety, Redlan. Heads from five different F_1 plants of the above cross with the segregating F_2 vitreous and floury kernels were used for this study. High lysine and normal sorghum grains from each head, along with parent Redlan and IS 11758 and another high lysine line, IS 11167, were used in the protein fractionation procedure. Kernels were

ground in a Wiley mill, defatted and then ground to a fine powder using a small ball mill. Protein was fractionated according to the Landry-Moureaux procedure as previously applied to sorghum. Lysine was determined on these ground samples by ion exchange chromatography.

The first fraction, representing the albumins, globulins and free amino acids soluble in sodium chloride solution, has the highest amount of lysine in comparison to other protein fractions, and any increase in its percentage distribution would naturally increase the percent of lysine in the whole kernel. The classes of proteins solubilized by isopropanol (Fraction II), and isopropanol containing mercaptoethanol (Fraction III), are referred to as kafirins and alcohol soluble glutelins respectively and these proteins have the lowest content of lysine. The kafirins and alcohol soluble glutelins average 52.9 percent in the normal sorghum as compared with 33.9 percent in the high lysine sorghum. This reduction in Fractions II and III accounts for part of the increase in the lysine content in the high lysine sorghum kernel. The fourth fraction which contains nitrogen soluble in borate buffer and mercaptoethanol, is similar in normal and high lysine sorghum. The fifth fraction contains nitrogen soluble in borate buffer, mercaptoethanol and sodium lauryl sulfate. This fraction increases in high lysine sorghum when compared with normal sorghum. Thus, the decrease in the percentage of soluble nitrogen in the second and third fractions, of high lysine sorghum is compared by a concomitant increase in the first and fifth fractions. The distribution patterns of high lysine Ethiopian lines IS 11758 and IS 11167 are similar to the average distribution patterns observed with the high lysine F_2 sorghum kernels obtained by crossing Redlan and IS 11758. Studies on the ratio

of normal to high lysine kernels on segregating heads indicate that the high lysine character is simply inherited and can be easily transferred by standard plant breeding procedures.

Zein synthesis in opaque-2 maize endosperms is reduced to about 50 percent of normal with a concomitant increase in the saline soluble and glutelin fractions. Since the protein distribution patterns obtained with high lysine sorghum are similar to those observed in opaque-2 maize, the gene action of these two mutants is probably similar.

Cereal Chemistry, 1975, 52:119-111.

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: (i) photoperiod insensitivity, (ii) 3-dwarf, (iii) broad agronomic adaptability, (iv) colorless pericarp, and (v) translucent endosperm. Selfed seed was treated with 0.1% diethyl sulfate (DES) for 3 hours to induce opaque endosperm mutants at 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 sporphytic 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/100g 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 vitrous 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 (IS11758), and the P721 opaque were compared in feeding trials with weanling rats and chicks. IS11758 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 IS11758 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 possesses 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
1975.

ABSTRACT

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

Seeds of two colorless pericarp, vitreous endosperm sorghum lines were treated with diethyl sulfate (1 ml. DES/100 ml. H_2O/3 hrs) to induce opaque endosperm mutants. Both parent lines are photoperiod insensitive. Approximately 500 putative opaque mutants were selected over a light box from M_3 seeds on each of 23,000 M_2 heads 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 for protein and lysine concentration. Several opaque endosperm mutants with significant increases in lysine were identified. These mutants fell into two classes. Class I opaque mutant seeds produced normal plants with no obvious evidence of sporophytic abnormalities. Class II opaque mutant seeds were associated with pleiotropic sporophytic effects ranging from complete lethality to dwarfs and chlorophyll deficiencies. One high lysine opaque endosperm mutant in Class I is of particular interest. Mutant P-721 has plump kernels with an average whole grain lysine content of 2.8 (g/100 g protein) at 17.5 percent protein relative to the normal sib average of 1.7 percent lysine at 15.4 percent protein. Inheritance studies suggest partial dominance of the opaque character.

Agronomy Abstracts, 1974, p. 66

ABSTRACT

PROTEIN QUALITY IMPROVEMENT IN GRAIN SORGHUM.
D. P. Mohan and J. D. Axtell.

The protein quality of sorghum grain is limited by the low lysine content of the mature grain, which reflects the high prolamine content of the endosperm. Two sources of sorghum germplasm which improve protein quality are now available. First, a gene which reduces prolamine content is present in the genome of two floury endosperm varieties from Ethiopia (IS11758 and IS11167). Second, another high lysine gene (P-721) has recently been identified among M_3 progeny of seeds treated with the chemical mutagen diethyl sulfate. Rations produced from both sources of high lysine germplasm have substantially improved biological value in monogastric feeding experiments.

Proceedings of the International Sorghum
Workshop, Mayaguez, Puerto Rico.
January 1975 (In Press)

ABSTRACT

IDENTIFYING GRAIN SORGHUM VARIETIES WITH A TESTA AND LOW CONCENTRATIONS OF TANNINS. D. L. Oswalt and J. D. Axtell.

A group of Sorghum bicolor (L.) Moench lines possessing a partial or complete testa containing a low concentration of tannins was identified by using the VHCl and MVHCl tests to estimate catechin equivalents (CE) in grain samples. Sample group I (low CE for the VHCl and the VMCHl tests) was without a testa, group II (Low CE for VHCl and high CE for VHCl tests) possessed a partial or complete testa, and group III (High CE for both the VHCl and MVHCl test) all had a testa. Three near isonitrogenous lines with similar concentrations of lysine, were selected from each group. Each sample was supplemented with 2% vitamins, 4% minerals and 0.8% lysine-HCl fed for 28 days to weanling male rats. Weight gains per rat of 78g, 84g and 40g and feed efficiency ratios of 4.2, 4.1 and 6.4 for mean values of the three selected lines in group I, II, and III respectively, verified that group II-type lines having the advantage of partial or complete testa did not contain levels of tannins which significantly reduced rat weight gains as biological estimates of protein quality.

Agronomy Abstracts, 1974 p. 107

ABSTRACT

MANAGEMENT PRACTICES AND FACTORS LIMITING SORGHUM YIELDS IN LOW PRODUCTION SITUATIONS. D. L. Oswalt.

Sorghum is grown in a wide range of generally adverse soil, climate, and management situations, chiefly as a food crop in low yield areas. The evolvement of sound, secure, productive, community crop management alterations, with visible improvement in the social well-being of all the people involved, and with no initial risks or costs makes the task very difficult, but such are the initial requirements to insure actual long-term yield improvement. The total community must be involved, otherwise the community will return to the traditional practices once an innovator leaves the area. However, introduction of genotypes that will respond to more fertile soil areas are resisted by the subsistence farmer because he cannot actually work the heavier, more difficult soils with the tools and power that he has available. Sorghum technology is presently developed for low production areas to where yields have been doubled in demonstration plots, but the adjacent farmer has not accepted what he has seen others do. It is; therefore, essential that: a) localized techniques and production supplies to insure stable yields be identified, proven and provided, b) that responsive genotypes with resistances to diseases, pests and adversities of the local environment be provided which are of good quality and which are palatable to the consumers, c) that storage, distribution, and utilization facilities be obtained, and d) that the producer in his social environment be accepted and advanced by the increased products of his efforts as dictated by his felt needs, if sorghum production in low production areas is to be significantly and permanently increased.

Proceedings of the International Sorghum Workshop,
Mayaguez, Puerto Rico. January 1975 (In Press)

ABSTRACT

ESTIMATING THE BIOLOGICAL EFFECTS OF TANNINS IN GRAIN SORGHUM.
D. L. Oswalt.

The CE estimates of tannins by the VHCl procedure and the MVHCl procedure can be used to predict the influence of tannins on rat weight gains. These two tests can be used to divide sorghum varieties into low tannin groups with and without a testa and a high tannin group for breeding program selections and selections for comparisons of nutritional qualities of sorghum varieties. The VHCl tests appeared to be the best single test for predicting biological values other than the IVDMD procedure. The identification of varieties (Group II types) which have a testa and low levels of tannins when mature, or which have tannin levels which are reduced in early storage, may provide varieties which have partial bird and weathering resistance and not reduce protein availability, but even low levels of tannin in grain samples will reduce the potential availability of proteins for animal growth. It is important that breeding and production programs recognize the presence or absence of tannins in selections being made or produced for utilization as feeds or foods.

Proceedings of the International
Sorghum Workshop, Mayaguez, Puerto
Rico. January 1975 (In Press)

ABSTRACT

IMPROVED PROTEIN QUALITY IN CEREAL CROPS: PRESENT UTILIZATION AND FUTURE POSSIBILITIES. D. L. Oswalt

The identification of high lysine maize in the early 60's has resulted in intensive research in other cereal crops for similar lysine controlling mechanisms to improve cereal protein quality. Widespread production of the high lysine varieties which show two- to three-fold increases in nutritional quality has been slow due to modifier genes that lower their yields. However, geneticists are making major progress in the development of high lysine varieties that measure up to a normal yield potential with other characteristics that will help reduce production risks for commercial and subsistence farmers. Improved varieties of maize, sorghum, barley, triticale and other cereals will have an increasing impact on rural subsistence farming areas and on whole populations that depend on cereal grains for over 50% of the protein in their daily diet. As the population of the world increases the demand will be even greater for improved quality cereal protein in the years to come. High lysine feed grain for meat production from monogastric animals will also be required to satisfy the increasing demands for protein.

PROCEEDINGS of The World Population Society
Meetings, Washington, D. C.
February 7, 1974 (In Press)

ABSTRACT

THE BROWN MIDRIB GENE (brm)* IN SORGHUM AND ITS AFFECT ON FORAGE QUALITY.
K. S. Porter, J. D. Axtell, V.L. Lechtenberg, and V. F. Colenbrander.

Workers with brown midrib mutants in maize have shown that of the four mutants known, the bm_3 gene causes the greatest decrease in percent lignin and the correlated largest increase in percent *in vitro* dry matter digestibility (IVDMD), when mutant and normal tissues were compared.

Animal feeding trials with bm_3 and normal corn stover silage revealed significant increases in average daily gain, feed efficiency and daily dry matter intake with the bm_3 material when compared to a commercial hybrid.

In this study seed of two sorghum lines was treated with diethyl sulfate to induce mutations. Nineteen separate brown midrib mutants were identified from segregating M_3 head rows. Mutant plants had characteristic brown pigmentation in the leaf midrib on the back side of the leaf, in the stem behind the leaf sheath, in the culm, in the immature panicle branches, and in the stem pith. Pigmentation was most obvious in new tissue and in some cases faded slightly as tissue matured.

Fiber analysis showed differences among the mutants in the magnitude of the decrease in percent lignin associated with the mutant character. *In vitro* tests showed an apparent correlation between decreased lignin content and increased IVDMD percentage. It is probable that several different *brm* loci are present in sorghum, but allelism tests have not been completed.

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* *brm* is the tentative gene symbol.

Proceedings of the International Sorghum
Workshop, Mayaguez, Puerto Rico.
January 1975 (In Press)

Samples of News Releases and Other Articles Related to Significant Research of this Project follow:

NORTHEAST-MIDWEST — THE POULTRY TIMES — AUG. 18, 1975—10

Purdue Scientist Finds

High Lysine Grain Sorghum Shows Superiority Over Normal Grains

PULLMAN, Wash. — Nutritional quality of a Purdue naturally occurring high lysine sorghum grain (IS11758) and a chemically induced high lysine mutant (P-721 Opaque) showed marked superiority to that of normal sorghum grains in a growth study with day-old chicks.

This was reported by Dr. W.R. Featherston, Purdue animal scientist, at the 64th annual meeting of the Poultry Science Association at Washington State University. The animal scientist and colleagues found the improved performance solely attributable to the high lysine content in the sorghum.

"When the sorghum grains were compared in diets in which all of the protein was supplied by the sorghum grain," he explained, "the high lysine sorghums IS11758 and P-721 Opaque produced weight gains which were approximately three and two times, respectively, those noted with normal sorghum." He said approximately 40-50 per cent less feed was required per unit gain.

When the normal sorghum diet was supplemented with lysine to equal the level supplied by the high lysine sorghum (IS11758), the results were similar chick growth and feed efficiency.

High lysine sorghum (IS11758) was also compared on an isonitrogenous basis with normal sorghum in 15 per cent protein diets, with the non-sorghum protein provided by safflower or soybean meal, he said.

"Chicks fed the high lysine sorghum diets supplemented with safflower meal or soybean meal [illegible] 3.0 times [illegible] and were [illegible] efficient in [illegible] utilization of their [illegible] corparts fed normal [illegible] sorghum diets," Featherston [illegible].

Working with Featherston on the research were Dr. J.C. Rogler, nutritionist, and Drs. [illegible] and D.P. Molnar, geneticists.

Journal and Courier, Tues., Aug. 19, 1975

DR. ROY FEATHERSTON

Purdue nutritionist wins award

Dr. Roy Featherston, a poultry nutritionist at Purdue University, received the 1975 American Feed Manufacturers Association award at the Poultry Science Association meeting in Pullman, Wash.

Dr. Featherston, at Purdue since 1963, received the award for distinctive work demonstrating sound work in poultry nutrition, according to association officials.

He was cited for his discovery that methionnine supplementation is a practical means of utilizing bird-resistant sorghum grain in poultry rations.

He received a $1,000 check and an engraved plaque from Rex Parsons, an AFMA official.

Dr. W. J. Stadelman, also of Purdue, received the American Egg Board's Egg Science Award recognizing him for his research in usage of eggs by Indiana institutions.

Journal and Courier
Farming Report
Nov. 15, 1974
B-3

Share in development

Dr. John D. Axtell (left), Purdue University geneticist and sorghum project director, views heads of a new chemically induced high lysine sorghum mutant held by Dr. D. P. Mohan, doctoral student in agronomy at Purdue. Mohan, a native of India, working in cooperation with Axtell, developed the mutation which is readily adaptable to temperate growing regions and as high in protein quality as two Ethopian high lysine lines announced last year.

article follows on next page....

Discovered at Purdue

New sorghum line to boost production

Development of a chemically induced high lysine sorghum mutation that rivals Mother Nature's offerings may boost future commercial use of this crop in the United States.

Disclosure of the improved protein quality mutant sorghum came today at the American Society of Agronomy's 66th annual meeting in a paper delivered by D. P. Mohan, Purdue University doctoral student in agronomy and a native of India.

This follows by only 13 months announcement of the discovery of two Ethopian sorghum lines containing twice as much lysine as normal sorghum. Dr. Rameshwar Singh, fellow countryman of Mohan, participated in that discovery, also made at Purdue.

Working in cooperation with Dr John D. Axtell, Purdue geneticist and sorghum project director, both men did doctoral research with sorghum.

"Because of delayed flowering, the two Ethopian lines are not immediately useful in temperate areas," Mohan reported to the Society. "But the chemically induced high lysine mutant (labeled P-721) has great promise for use in such climates within the next few years.

Protein quality of the two Ethopian lines and the "man-made" mutant is nearly the same, Mohan pointed out. "Protein averages about 12 per cent in normal sorghum," he said, "14-15 per cent in the chemically induced mutant, and 16-17 per cent in the Ethopian lines.

'But P-721 has two principal advantages over the African lines as far as use in the United States is concerned," Mohan added. "First, it stems from a U.S. type sorghum, consequently is readily adaptable to temperate sorghum growing areas. Secondly, the kernels have better agronomic characteristics. The P-721 kernels are plump whereas the kernels of the Ethopian lines are dented. Dented kernels do not normally produce as high a yield.'

Another important characteristic of the chemically induced mutant is its white seed color. This becomes important where sorghum is used directly as a human food (as in African and Asia) in the daily diet, for white is preferred. Seeds of the Ethopian lines, although sweet and tasty, are orange in color.

Mohan examined some 23,000 sorghum heads and selected 500 "possible" mutants for chemical analysis in his research. From this group, P-721 proved superior as a chemically induced high lysine mutant. The high lysine content of this line is inherited and can be easily transmitted to other varieties by conventional cross-breeding techniques.

"In a 28-day feeding trial, weanling rats fed the P-721 opaque sorghum grain gained three times as much as those fed normal sorghum," Mohan said. The weanling rat trial is standard procedure for evaluating nutritional quality of cereal grains.'

Sorghum is the fourth most important cereal crop in the world, topped only by wheat, rice and corn. In the U.S. and Latin America it is produced chiefly as livestock feed.

Singh, who has returned to India, screened some 9,000 varieties in the world sorghum collection before discovering the high lysine gene in the two Ethopian lines. Mohan, who will receive his doctorate in December, took a different route, trying to arrive at the same goal — a better protein quality sorghum. The new mutant, of course, did not appear in the world sorghum collection.

The sorghum research project at Purdue is in cooperation with the U.S. Agency for International Development.

Mohan, who received the undergraduate degree from Panjab University and masters degree from Delhi University, India, came to this country with his wife in 1969.

For the
PRESS

Agency for International Development
OFFICE OF PUBLIC AFFAIRS

FOR IMMEDIATE RELEASE
WEDNESDAY, NOVEMBER 13, 1974

AID-74-81
Contact: Jerry Rosenthal
(202) 632-9309

AID-PURDUE PROJECT PRODUCES NEW SORGHUM WITH IMPROVED QUALITY OF PROTEIN

Purdue University researchers, working under a grant provided by the Agency for International Development, have produced another variety of sorghum that may help improve the diets of millions of the world's hungriest people.

Disclosure of the improved protein quality mutant sorghum came today (November 13) at the American Society of Agronomy's 66th annual meeting in Chicago. D. P. Mohan, a native of India and a Purdue doctoral student in agronomy, delivered the paper.

Dr. Mohan's report follows by only 13 months the announcement of the discovery of two Ethiopian sorghum lines containing twice as much lysine as normal sorghum. Lysine is one of the important amino acids, the building blocks of protein, and essential to good nutrition.

The new high lysine mutant is chemically induced, while the Ethiopian varieties were found growing naturally. Both, however, are considered nearly equal in protein quality. The new mutant (labeled P-721), however, apparently thrives best in temperate climates, while the Ethiopian varieties are tropical.

Dr. John Axtell, Purdue geneticist and sorghum project director, said, however, that the new mutant also had great possibilities for tropical and sub-tropical climates, where sorghum is a basic food for some 500 million people of Asia and Africa.

"This gives us another tool to work with, " Dr. Axtell said." We have crossed it with tropical types, including the Ethiopian strains, and are continuing to work with it for use in tropical as well as temperate climates."

He said the seeds of the new mutant have been sent to research stations in India, the Middle East and Mexico for testing and crossing under varied conditions. The high lysine content of P-721 is inherited and can be easily transferred to other varieties by cross-breeding.

More...

Dr. Axtell added that he believed the new variety would be planted extensively within a year in the United States and thus would be available for shipment overseas.

Sorghum is the world's fourth most important cereal, after wheat, rice and corn. However, the nutritional quality of most sorghum varieties is poor, as compared to that of other major cereals.

In an effort to learn if the nutritional content of sorghum can be improved, AID launched the sorghum research project in 1966. The Agency has provided $1.7 million to Purdue University to carry on the research. The discovery of the two high-lysine, high-protein Ethiopian lines was announced in October, 1973 after thousands of varieties from all over the world had been classified and tested.

The new P-721 mutant, derived from a U.S. type, was selected after Dr. Mohan had examined 23,000 sorghum heads. From these he chose 500 "possible" mutants for chemical analysis in his research. Out of this group, P-721 proved superior as a chemically-induced high lysine mutant.

"In a 28-day feeding trial, weanling rats fed the P-721 opaque sorghum grain gained three times as much as those fed normal sorghum," Dr. Mohan said. The weanling rat trial is standard procedure for evaluating nutritional quality of cereal grains.

In his paper, Dr. Mohan noted that the P-721 kernels are plump, whereas the kernels of the Ethiopian lines are dented. Dented kernels, he said, do not normally produce as high a yield.

Another important characteristic of the chemically induced mutant is its white seed color, Dr. Mohan reported. White sorghum is preferred in most of the areas where sorghum is a basic food. Seeds of the Ethiopian lines, although sweet and tasty, are orange in color.

# # # #

Agriculture

A significant breakthrough occurred in 1973 with grain sorghum, the fourth largest food crop in the world and the basic cereal grain in many parts of Asia and Africa. Two new strains of sorghum that are high in protein were identified by John D. Axtell and Rameshwar Singh of Purdue University. The new strains contain 30 to 40 per cent more protein than other varieties, which should bring new hope to the 300 million protein-deficient people in the world. They also contain twice as much lysine, a substance that increases digestibility. The tendency to store more protein and lysine is carried in the genes of the plants, and can be cross-bred into other varieties of sorghum all over the world.

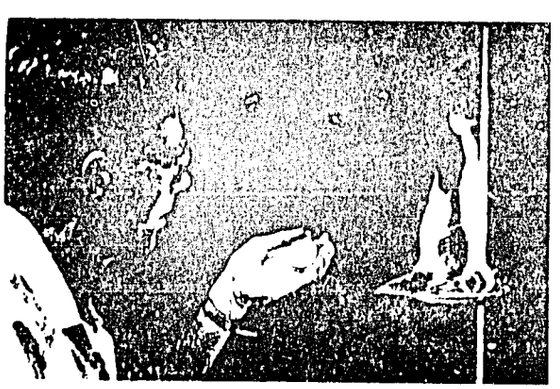
There were also noteworthy improvements in rice, the number one world food crop. A variety of rice plant that discourages birds from eating the growing grain was developed by scientists at the International Rice Research Institute at Los Baños in the Philippines. The new variety has sharp pointed flag leaves that protrude well above the point at which the grain forms. Another variety, IR-26, is resistant to brown plant hoppers and most other insects and diseases found in tropical Asia.

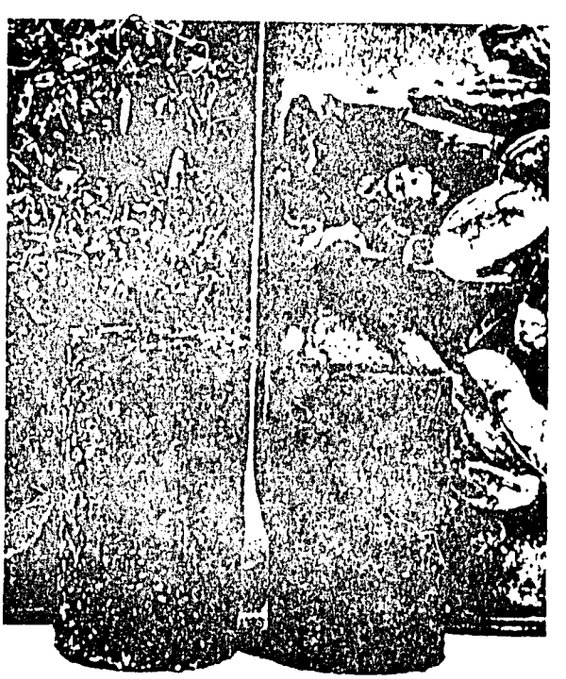
New crop varieties. Many new crop varieties with colorful and descriptive names were introduced in 1973 and 1974. These included: Tangi, a high-quality, high-yielding strawberry; Magnifico, a superior globe artichoke with rose-colored leaves; Self Blanche, a cauliflower in which the leaves naturally fold over the head so they do not have to be tied by hand; Sonnette, a new disease-resistant bell pepper; Vermilion and Caro-Red tomatoes; and Tamu-Triple, the first pickling cucumber formed by crossing three plants.

Other new varieties include Cherokee and Comanche erect blackberries, adapted for mechanical harvesting; Spartan Sleeper, an onion that will not sprout in storage; Vogue, a large, shiny dark red cherry; and Sparlett, a new pear with high blush that ripens 12 days ahead of the common Bartlett variety.

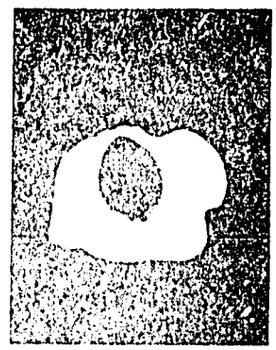
New planting methods. Many crops were planted without tillage during 1973 and 1974. That is, they were planted

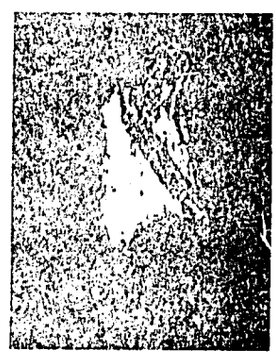
Chemical engineer William Albrecht tests a sample of a new fireplace log made of peanut shells and wax against a traditional wood chip and wax log, *below*. The new material burns brighter and longer, and puts peanut wastes to good use. Pressed logs, *right*, stand in front of their respective ingredients





Purdue University plant researchers Dallas Aswalt, Rameshwar Singh, and John D. Axtell, left to right, hold a high-lysine sorghum they developed. Cross sections of the new seed, *right*, and a normal one, *below right*, show that the high lysine seed has a floury endosperm (white) characteristic that provides it with a higher protein content.





Agriculture
Continued

directly on land that was not plowed, disked, harrowed, or cultivated. A herbicide was used to kill weeds and sod before the seeds were drilled into the soil. The technique is of worldwide significance because it conserves the soil, water, and energy. In the United States, an estimated 5.5 million acres of crops were planted in this way in 1974. This was an increase of about 12 per cent over the amount of acreage planted by this technique in 1973.

Mechanization. Substitution of machines for labor continued. Clarence M. Hansen and Richard Ledebuhr, of the Michigan Agricultural Experiment Station at East Lansing, designed a foolproof electronic sensing device that selects processed cherries which have not had their pits removed. Therefore H. Short, an agricultural engineer at Ohio State University, developed a machine that harvests greenhouse lettuce.

The sweet cherry harvest in Michigan in 1973 and 1974 was completely mechanized. A chemical ethephon was sprayed on the trees a few days before the harvest to loosen the cherries. Then machines dislodged the cherries by shaking the trees, repeatedly, with little tree injury and no damage to the fruit.

Nitrogen fixation. Ralph W. F. Hardy and E. D. Hare, of the E. I. du Pont de Nemours Experiment Station Laboratories in Wilmington, Del., achieved a nearly sixfold increase in nitrogen fixation with field grown soybeans. Nitrogen fixation is the process by which bacteria that live in knobbed growths along a plant's roots turn atmospheric nitrogen into a chemical form of nitrogen that can be used by the plant.

The scientists found that increased photosynthesis, which they achieved by raising the carbon dioxide levels around the leaves, produced more carbohydrates in the plants, which in turn increased the amount of nitrogen that the bacteria processed. The carbon dioxide around the leaves was raised from a normal of about 300 parts per million to 1,000. They used specially open-top plastic structures that trapped some of the added carbon dioxide so it could be measured by infrared gas analyzers. Since soybeans do not generally respond to nitrogen fertilizers, this discovery sets the stage for