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ANNUAL REPORT

ON

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

April 1, 1977 - September 30, 1979

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prepared by:

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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, West Lafayette, Indiana 47907.

3. Contract Period: April 1, 1975 to September 30, 1979

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B. The Ethiopian high-lysine gene is associated with an 80% increase in the lysine concentration of whole sorghum grain. It is generally accepted that the gene reduces yield in all genetic backgrounds. We believe that the Ethiopian high-lysine gene, in its native background genotypes, has greatest applicability as a special purpose grain for people requiring a high-protein food source, i.e. pregnant and lactating women and weaned children, as an alternative to externally supplied supplements. The chemically induced P-721 opaque gene is associated with a 60% increase in the lysine concentration of whole grain in isogenic comparison with the sib

line. The lower rate of dry matter accumulation during development on a per seed basis confirms that this gene also is associated with reduced seed weight. However, selection experiments indicate that yield component compensation, particularly in seed number, allows high yielding P-721 opaque lines to be developed. The increment in lysine concentration contributed by the gene in high-yielding genetic backgrounds may not be as great as in the isogenic line.

When seven varieties of sorghum grain varying in tannin content were fed to chicks, a high negative correlation (r = -0.92) was observed between tannic acid equivalents (TAE) of the grain and growth rate. An identical correlation (0.92) was noted between TAE and feed efficiency (grain/feed). Supplementing a high tannin sorghum (HTS) diet with ferric chloride (0.5 or 1.0%) to enhance tannin oxidation failed to overcome the detrimental effects of tannin. Similarly, the addition of diethyldithiocarbamate (0.1 or 0.2%) to a HTS diet to inhibit polyphenol oxidase, and thus reduce tannin oxidation, did not affect the tannin toxicity. Chicks fed HTS diets produced significantly more excreta while consuming less feed than chicks fed low tannin sorghum (LTS) diets. Hence, dry matter utilization of chicks fed HTS was significantly poorer than chicks fed LTS. The growth depression produced by feeding HTS in a sorghum-soybean meal diet could be completely overcome by methionine supplementation. However, the addition of methionine had no effect on dry matter utilization. These results suggest that the growth depression associated with feeding HTS cannot be explained on the basis of reduced digestibility. Since the reduced feed efficiency observed with HTS is not completely corrected by methionine supplementation, lower digestibility appears to be the cause of the poorer feed efficiency.

Polyphenolic materials such as tannins constitute a natural defense mechanism which in grain sorghum provides protection against weathering, pre-harvest germination, and bird depredation. In some areas these stresses are so severe that low tannin types are difficult to produce. The agronomic advantages of high tannin sorghums are somewhat offset by the nutritional disadvantages associated with these types, which give relatively low weight gains and feed efficiencies in feeding trials with rats and chicks. A major incentive for our work is the resolution of this conflict between sorghum producers and feeders concerning the presence and effects of tannin in sorghum.

When we began to investigate the occurrence and effects of tannins in sorghum we found that the available techniques for quantitating the tannins were not satisfactory. We have therefore developed, or modified from previous techniques, a series of tannin assays which are more suitable. These assays are somewhat independent of each other because they measure different chemical properties of the tannins. Collectively, therefore, they serve not only to determine the amount of tannin present, but also to characterize it to some extent.

The vanillin assay has been widely used for sorghum tannin, at least partly because of its specificity for the flavanol type units of the condensed tannin polymers such as are present in sorghum. We have made several modifications of this assay which increase its accuracy, reproducibility and convenience, Perhaps the most significant change is the inclusion of a reagent-free blank which corrects for background absorption. We have shortened the extraction time from 24 hours to 20 minutes, and altered the reaction conditions. The reaction is quite temperature sensitive so the temperature must be controlled. Catechin,

although often used as a standard in this assay, gives non-linear standard curves and much less absorption than equivalent weights of tannin. Purified tannin should be used as standard whenever possible.

We developed a rapid and convenient technique, the Prussian Blue assay, which measures total extractable phenolics. This assay can be adapted for rough estimates of tannin content in the field or at the elevator, or for precise measurements in the laboratory. Non-tannin phenolics in sorghum cause little interference in high tannin varieties. Even this low level of color due to non-tannin phenolics can be largely accounted for and subtracted out by comparing values obtained by extracting with water and with a salt solution. The salt seems to prevent tannin from extracting without interfering with the extraction of other phenolics, so that the difference in values obtained with water and salt solution extraction are due to tannin.

Perhaps the most characteristic property of tannins is their propensity for interacting with proteins; this may largely account for their biological properties. We have devised assays which measure, under standard conditions, the protein precipitating power of tannins. The amount of tannin in the precipitate is determined spectrophotometrically after redissolving the pellet. The amount of protein precipitated is measured using ^{125}I-labelled protein such as serum albumin. These assays are particularly useful for studying tannin-protein interactions. Depending on the conditions, purified sorghum tannin can precipitate up to six times its own weight of serum albumin. The protein precipitating power of high tannin sorghum can be assessed in the ground grain without any extraction or other treatment which might modify or fractionate the tannins, by mixing constant amounts of sample with increasing

concentrations of ^{125}I-albumin. After centrifugation, measurement of unbound ^{125}I in the supernatant gives a titration curve which shows the protein precipitating capacity of the grain at saturation.

These assays, when applied to mature sorghum grain, all give good correlations with nutritional quality as assessed by rat and chick feeding trials measuring weight gain and feed efficiency. Preliminary studies with immature grain show a much poorer correlation between the results of chemical assays and feeding trials. The reasons for this apparent discrepancy are under investigation.

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 world-wide 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 further improve 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 is 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 AT 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

The objectives are currently being pursued under the Title XII Sorghum and Millet CRSP.

D. RESEARCH ACCOMPLISHMENTS

Summary reports of the major research accomplishments are included in the following sections. (See list in Table of Contents.)

CURRENT STATUS OF PROTEIN QUALITY IMPROVEMENT IN GRAIN SORGHUM

J. D. Axtell, S. W. VanScoyoc, P. J. Christensen and G. Ejeta
Department of Agronomy

Introduction

Sorghum is an important crop species for the production of grain for human consumption in Africa and Asia. The grain provides an important source of carbohydrate and is similar to other major cereals as a source of total protein. The protein quality, however, is relatively poor in comparison with that from other major cereal grains. Mertz *et al.* [1] estimated that the biological value of sorghum grain is poorer than that of any other major cereal, primarily because of the low lysine content of the sorghum endosperm storage proteins. Two genetic mutants, one naturally occurring and one induced, have been identified that increase the lysine content of the sorghum endosperm and improve the protein quality of the grain. The purpose of this paper is to briefly review the origin of these mutants and describe the recent results of experiments on the relationship between improved protein quality and total grain production in sorghum.

Ethiopian High-Lysine Gene

Singh and Axtell [2] screened about 10,000 entries in the World Sorghum Collection and identified two floury endosperm varieties from Ethiopia that contained a gene that significantly increased the level of protein and also increased the lysine concentration of the endosperm

proteins. The screening process involved cross-sectioning seeds from each entry to identify those with floury endosperm phenotypes and then evaluating grain samples from those selected entries for protein and lysine concentration. Sixty-two floury endosperm lines were identified, of which two (IS-11167 and IS-11758) had a significantly higher lysine content than normal sorghum. These lines contain approximately 15 - 17% protein in comparison with normal checks averaging about 12% protein. The lysine content of the Ethiopian high-lysine selections is approximately 3.1% (expressed as per cent of protein) and 0.50% (expressed as per cent of sample) in comparison with normal sorghum values of 2.0 and 0.26%, respectively [3]. The biological value of the Ethiopian high-lysine grain is also significantly higher than normal sorghums in isonitrogenous rat feeding experiments [2]. It has been established that the concentration of alcohol-soluble proteins is significantly reduced in high-lysine endosperm, relative to values present in normal sorghum endosperm [4,5].

Utilization of High-Lysine Sorghum in Ethiopia

A collection trip was made in 1973 to determine whether the high-lysine varieties identified in the World Collection were being cultivated by farmers in Ethiopia. The lines originally identified from the World Germplasm Collection were obtained in Wollo Province in the central highlands of Ethiopia. Farmers continue to grow these varieties in mixed plantings of sorghum varieties in this area of Ethiopia. A large number of varieties similar to the original high-lysine variety were collected in addition to an equivalent number of normal varieties for comparative purposes. Ejeta [6] has evaluated the protein and lysine content of grain from high-lysine and normal varieties grown under actual

field conditions in Ethiopia. Figure 1 illustrates the lysine and protein concentration in this series of high-lysine and normal sorghum varieties. The mean lysine concentration, expressed as percent of protein, was 2.88 for the high-lysine entries and 2.17 for the normal sorghum varieties grown in the same environment. Protein values were 15.7 and 11.4%, respectively. It seems likely that the high-lysine gene has been present in Ethiopia for a long period of time, since there is great diversity in panicle morphology, maturity and plant height among the high-lysine genotypes collected. The farmers roast the heads of the high-lysine varieties in the late dough stage and eat the grain in mixtures with grain from normal sorghum varieties prepared in a similar way. There is general recognition by the farmers that the yield of high-lysine varieties is significantly less than normal varieties. The reason given to us for growing these varieties is that the high-lysine grain has superior flavour qualities and improved palatability.

There is a good opportunity to utilize these high-lysine varieties in African countries as high-protein, special-purpose sorghum varieties. The protein concentration is increased by about 30%, along with the significant increase in protein quality. The grain from these varieties is recognizably different for marketing purposes because of the somewhat dented kernel phenotype of the mature grain. The flavour characteristics also appear to make these varieties quite acceptable for human consumption. We propose that these Ethiopian hgih-lysine varieties should be utilized in rural populations as special-purpose sorghums for people who have a high protein requirement. It should be possible for farmers in rural areas to produce an adequate quantity of high-lysine sorghum grain for use as a weaning food and a supplement for pregnant women and nursing mothers on a

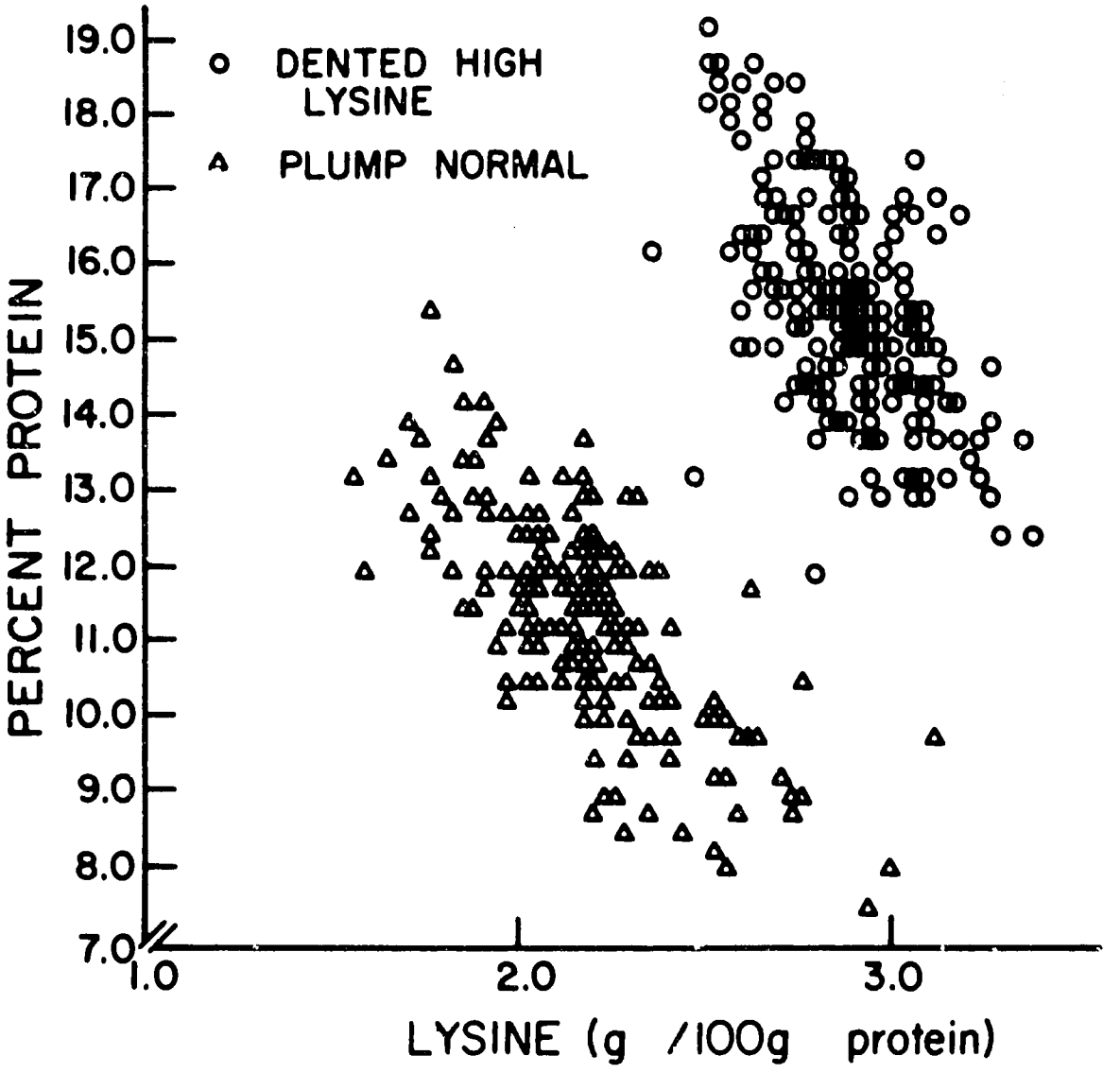

Figure 1 Relationship of protein and lysine in normal and high lysine sorghum lines collected from the same environment in Ethiopia. (From Axtell et al, 1978)

small section of their farm. It may also be possible to develop a marketing system whereby these grains can receive a market premium when sold in the cities. [Caution - Very recent studies by Graham at Johns Hopkins (personal communication to E. T. Mertz) suggest that sorghum flour is quite indigestible by infants and small children. Since this is contradictory to results with laboratory animals, more information is needed before high-lysine sorghums are utilized as weaning foods.]

Chemically Induced High-Lysine Mutant

Mohan [7] utilized chemical mutagenesis to induce a second high-lysine gene mutation in sorghum. The parent line used for the mutagen treatments was a photoperiod insensitive, three-dwarf sorghum line with relatively broad agronomic adaptability. The parent line also had a colourless pericarp and translucent (vitreous) endosperm so that progeny from the mutagen treatments could be screened for opaque mutant kernels over a light box. Selfed seed was treated with diethyl sulphate by soaking in a solution containing 1 ml DES per 1000 ml of distilled water for three hours. The M_1 plants were grown in Lafayette, Indiana, during 1972 and each head was bagged to ensure self-fertilization. M_2 plants were then grown in Puerto Rico during the winter of 1972-73 and each M_2 head was again bagged to ensure self-fertilization. Approximately 23,000 bagged M_2 heads bearing M_3 seeds were harvested in the spring of 1973 in Puerto Rico and shipped to Lafayette for evaluation.

Seed from each head was threshed and examined for opaque kernel segregates over a light box. A total of 445 putative opaque mutants were identified and seed from each segregating head was separated into vitreous and opaque classes. Both classes of seed from each putative mutant head

were then analysed for protein and lysine concentration. Of the 445 mutants, only 33 were identified that had an increase in lysine concentration greater than 50%. Plants from each of these 33 opaque and normal sib seed lots were grown in paired rows to evaluate them for any morphological changes associated with the change in endosperm phenotype. Most of the opaque mutants were found to drastically affect either plant or seed development. Only one of these 33 (P-721) was found to produce normally appearing plants and seeds. The P-721 opaque mutant produced an increase of about 60% in lysine concentration. It is a single gene that is simply inherited as a partially dominant factor. The biological value of P-721 grain is significantly higher in monogastric feeding experiments than normal sib counterpart grain.

VanScoyoc has examined dry matter accumulation during grain development to determine what effect the P-721 mutant has on grain yield potential. Figure 2 presents the mean seed weight per head of P-721 opaque and normal sib heads at periods ranging from 10-59 days after pollination in a space-planted population. It is evident from these data that there is no difference in dry matter accumulation until approximately 31 days after pollination. After 31 days dry matter accumulation in the P-721 opaque line levels off, whereas dry matter in the normal sib line continues to accumulate for an additional week, plateauing at 38 days after pollination. VanScoyoc has also examined 1000-seed weight during grain development and his data are presented in Table 1. Seed weights of the normal and opaque lines are similar at 31 days after pollination, but diverge at 38 days after pollination. At maturity kernel weight for the opaque line is reduced 11-14% relative to its normal counterpart. No reduction in seed number was observed, so the difference between the lines can largely be

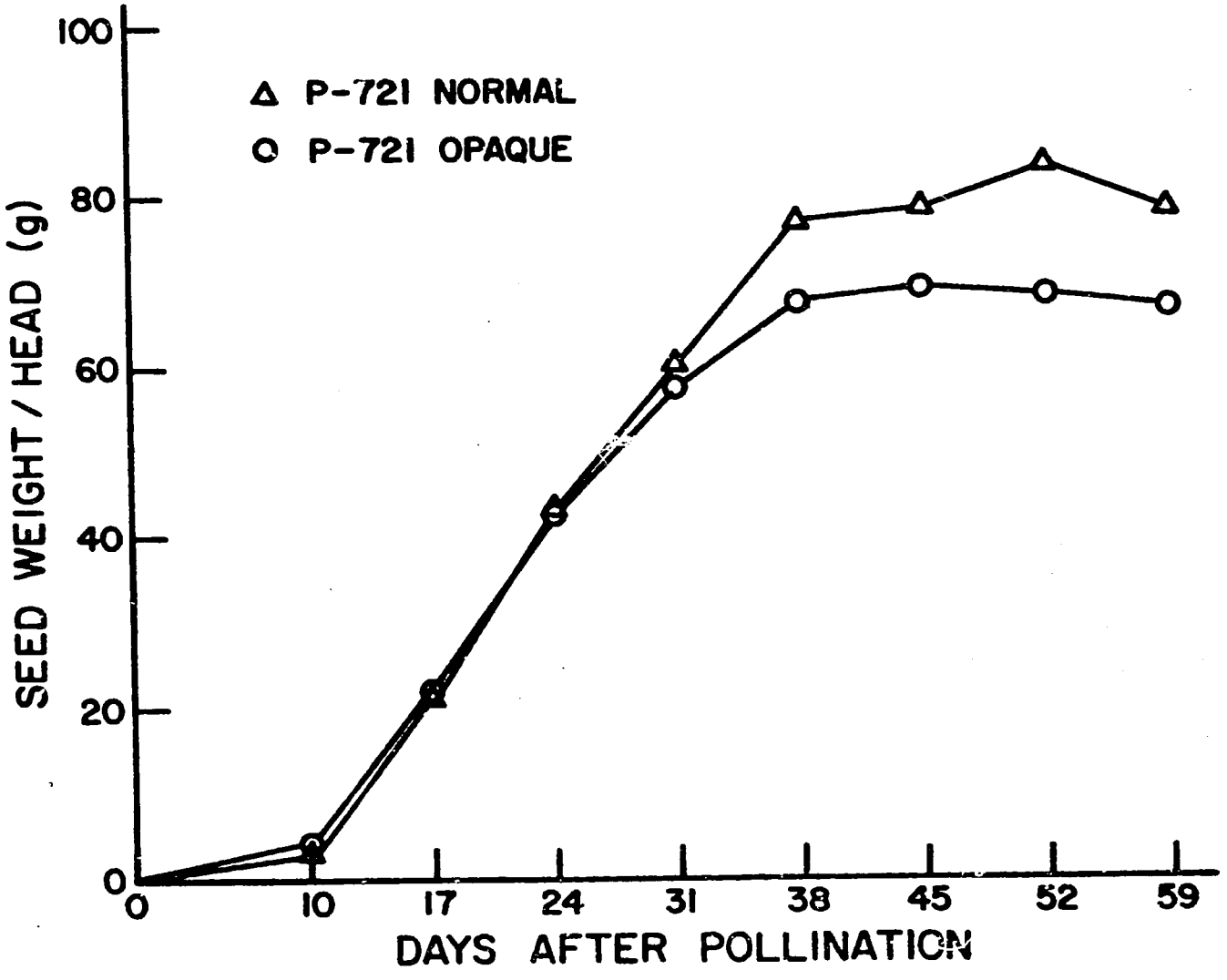

Figure 2. Mean increase in seed weight/head of P-721 opaque and P-721 normal sorghum (Sorghum bicolor (L.) Moench) during grain development in a space planted population.

TABLE I. MEAN 1000-SEED WEIGHTS OF P-721 OPAQUE AND ITS NORMAL SIB LINE DURING GRAIN DEVELOPMENT IN A SPACE PLANTED POPULATION

Days after pollination	1000-seed dry weight[a] P-721 normal	P-721 opaque	% P-721 opaque of normal
17	7.625	7.875	103.3
24	14.664	14.294	97.5
31	18.562	18.998	97.7
38	23.164	21.652	93.5
45	25.455	21.608	84.9
52	24.099	21.451	89.0
59	24.861	21.358	85.9

[a] Mean of three replicates.

accounted for by reduced kernel density. The reduction in kernel weight is in relative agreement with preliminary data from a 1977 four-replicate yield trial at 147 664 plants/ha showing a 9.4% total yield reduction for P-721 opaque compared with its normal sib line.

Yield of P-721 Derived Lines

Christensen [8] examined the grain yield of advanced breeding lines homozygous for the P-721 opaque gene in comparison with heterozygous and normal, homozygous vitreous sib lines.

The varieties used are F_4 derived bulks from crosses of P-721 by 22 unrelated high-yielding lines from the World Collection of sorghum varieties, and from Purdue University's sorghum breeding programme. The segregating material was handled as part of the regular breeding nursery. The population of inbred lines was selected for short to intermediate height, early flowering and general agronomic desirability. Opaques were selected in the F_3 and F_4 generations, largely on the basis of their acid orange 12 dye-binding capacity. The heads of the 378 lines included in the yield trial were checked for opaqueness by seed sectioning after harvest: 300 lines are opaque; 73 are segregating (indicating that the F_4 head had been heterozygous); and 5 are homozygous vitreous. The selected lines were tested in the F_5 in the two-block randomized complete block design at the Purdue Agronomy Farm during the summer of 1977.

The means for the three genotypes and four check varieties are given in Table II. Opaque lines yield marginally higher than the segregating lines from heterozygous heads, but the difference was not due to the opaque gene itself. This higher yield is almost certainly due to selection for high dye-binding capacity in the F_3 and F_4 generations among the

TABLE II. MEANS OF CHEMICAL AND AGRONOMIC TRAITS FOR COMPARISON OF GENOTYPES AT THE OPAQUES LOCUS FOR P-721 DERIVED LINES AND HIGH YIELDING CHECKS

	No.	DBC ($g \times 10^{-1}/l$)	Protein (%)	Yield ($kg \times 10^{2}/ha$)	100-seed weight (g/100)	Days to flowering	Height (cm)
P-721 genotype							
Opaque	300	7.64	12.51	46.58	2.50	72.8	135
Heterozygous	73	7.04	13.26	44.72	2.89	72.1	148.7
Normal	5	6.78	12.92	41.54	2.87	72.6	156
Checks							
954063 (Inbred line)	4	6.14	11.38	57.32	2.58	68.9	120
RS671 (hybrid)	4	6.18	11.66	56.35	2.30	65.0	111.2
NK300 (hybrid)	4	6.11	10.88	66.70	2.18	64.9	152.5

heterozygous and normal heads. To be selected for the yield trial, a heterozygous or a normal head would have to have had a very high protein percentage. Since high protein percentages are associated with low yield levels, one would presume that selection against yield occurred in the heterozygous and in the normal heads that were selected. The conjecture is supported by the high protein percentages of the segregating and vitreous lines in the trial. On the basis of this evidence, one would presume that P-721 derived opaque lines would not be higher yielding but should be as high yielding as their normal counterparts.

Seed weight of the opaques is significantly lower than the seed weight of the normal and of the heterozygous lines (Table II). The seed weight of the high-yielding checks is even lower than the seed weight of the opaques. In opaque-2 maize low seed weight is a problem because restricted ear morphology does not allow seed number compensation. Low seed weight is not closely associated with low yield in sorghum. The increased panicle size allows compensation for decreased seed weight by increased seed number.

Discussion

We conclude that the comparison of the yield of P-721 opaque with its isogenic normal sib line shows clearly that the grain-filling period of the opaque kernel is reduced in this particular genetic background. The resultant decrease in seed weight accounts for the reduction in grain yield in that background.

While it is possible that the P-721 opaque gene reduces grain-filling duration in many genetic backgrounds with a consequent decrease in seed weight, the yield data from the F_5 opaque and heterozygous advanced sib lines suggests that selection for yield will result in compensation in

number of seeds per head and number of heads per unit area. Appropriate genetic backgrounds can be selected that will significantly enhance yield potential of the P-721 opaque gene. The yield differential observed between isogenic opaque and normal lines in sorghum should not be a serious deterrent to the development of high-yielding sorghum varieties with improved protein quality, after the gene has been placed in the appropriate background.

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Final Report of the Research on Sorghum Tannins

Biochemistry Department, Purdue, April 1977 - September 1979
(Adapted from Abstracts of Reports
Presented at the Sorghum Utilization Conference, Wichita, 1979)

Polyphenolic materials such as tannins constitute a natural defense mechanism which in grain sorghum provides protection against weathering, pre-harvest germination, and bird depredation. In some areas these stresses are so severe that low tannin types are difficult to produce. The agronomic advantages of high tannin sorghums are somewhat offset by the nutritional disadvantages associated with these types, which give relatively low weight gains and feed efficiences in feeding trials with rats and chicks. A major incentive for our work is the resolution of this conflict between sorghum producers and feeders concerning the presence and effects of tannin in sorghum.

When we began to investigate the occurrence and effects of tannins in sorghum we found that the available techniques for quantitating the tannins were not satisfactory. We have therefore developed, or modified from previous techniques, a series of tannin assays which are more suitable. These assays are somewhat independent of each other because they measure different chemical properties of the tannins. Collectively, therefore, they serve not only to determine the amount of tannin present, but also to characterize it to some extent.

The vanillin assay has been widely used for sorghum tannin, at least partly because of its specificity for the flavanol type units of the condensed tannin polymers such as are present in sorghum. We have made several modifications of this assay which increase its accuracy,

reproducibility and convenience. Perhaps the most significant change is the inclusion of a reagent-free blank which corrects for background absorption. We have shortened the extraction time from 24 hours to 20 minutes, and altered the reaction conditions. The reaction is quite temperature sensitive so the temperature must be controlled. Catechin, although often used as a standard in this assay, gives non-linear standard curves and much less absorption than equivalent weights of tannin. Purified tannin should be used as standard whenever possible.

We developed a rapid and convenient technique, the Prussian Blue assay, which measures total extractable phenolics. The assay can be adapted for rough estimates of tannin content in the field or at the elevator, or for precise measurements in the laboratory. Non-tannin phenolics in sorghum cause little interference in high tannin varieties. Even this low level of color due to non-tannin phenolics can be largely accounted for and subtracted out by comparing values obtained by extracting with water and with a salt solution. The salt seems to prevent tannin from extracting without interfering with the extraction of other phenolics, so that the difference in values obtained with water and salt solution extraction are due to tannin.

Perhaps the most characteristic property of tannins is their propensity for interacting with proteins; this may largely account for their biological properties. We have devised assays which measure, under standard conditions, the protein precipitating power of tannins. The amount of tannin in the precipitate is determined spectrophotometrically after redissolving the pellet. The amount of protein precipitated is measured using ^{125}I-labelled protein such as serum albumin. These assays are particularly useful for studying tannin-protein interactions.

Depending on the conditions, purified sorghum tannin can precipitate up to six times its own weight of serum albumin. The protein precipitating power of high tannin sorghum can be assessed in the ground grain without any extraction or other treatment which might modify or fractionate the tannins, by mixing constant amounts of sample with increasing concentrations of ^{125}I-albumin. After centrifugation, measurement of unbound ^{125}I in the supernatant gives a titration curve which shows the protein precipitating capacity of the grain at saturation.

These assays, when applied to mature sorghum grain, all give good correlations with nutritional quality as assessed by rat and chick feeding trials measuring weight gain and feed efficiency. Preliminary studies with immature grain show a much poorer correlation between the results of chemical assays and feeding trials. The reasons for this apparent discrepancy are under investigation.

For maximum accuracy, pure samples of authentic sorghum tannin must be used as standards in tannin assays. In our laboratory, previously described preparations of sorghum tannin are contaminated with protein, which could significantly affect the properties of the tannin with which it is associated. We have devised a purification scheme for sorghum tannin which reduced protein contamination to low levels ($\leq$ 2%).

Several approaches to the problem of antinutritional effects have been considerod. Our colleagues at Purdue have removed the tannin from sorghum by extraction with aqueous alkalies or by milling off the surface layers of the grain, but the loss of nutrients which occur with these treatments are significant and probably unacceptable. We have investigated the possibility that sorghum tannins might be modified <u>in situ</u> to forms with reduced antinutritional properties. We have found that sorghum tannins are

readily converted, on addition of small amounts of aqueous ammonia to whole grain at room temperature and atmospheric pressure, to forms which do not respond to any of our chemical assays for tannin. Moreover, feeding trials of this treated high tannin sorghum show that the nutritional quality is correspondingly improved by the treatment, with weight gains on treated high tannin grain equivalent to those observed with low tannin sorghum. The treatment is not specific for ammonia; similar "detoxification" effects have been observed with several dilute aqueous alkalies, including an extract of wood ashes. Both moisture and alkali are required; a variety of heat treatments and conventional cooking procedures have been examined but none showed significant detoxification.

Detoxification of high tannin sorghum, no matter how simple the process, may not be the most desirable long-term solution to the tannin problem. Our goal is a genetic solution: sorghum varieties which have tannins and are thus agronomically superior, but which do not exhibit the antinutritional properties of tannin. During seed maturation some sorghum varieties have quite high tannin levels which diminish on ripening. In the Group II sorghums described by Cummings and Axtell, tannin content diminishes at maturation to undectable levels, unless special acidic solvents are used to extract the tannin. With Roger Bullard and W. C. Royall of the Denver Wildlife Service, we are examining the bird repellancy, nutritional quality, and other characteristics of selected Group II sorghums throughout the maturation process. Preliminary studies indicate that the tannin of immature grain may have properties somewhat different from that of mature grain. Brief boiling or freezing of immature sorghum followed by drying at room temperature converted the tannin to non-extractable forms, whereas the untreated but similarly dried controls

had extremely high amounts of extractable tannin. These treatments had no apparent effect on the nutritional properties of the tannin. The conversion of tannin to non-extractable forms by these treatments may be due to disruption of tannin-containing structures, permitting cellular components to bind tannins.

EFFECTS OF SORGHUM GRAIN TANNINS ON NUTRITIVE VALUE

J. C. Rogler and W. R. Featherston
Department of Animal Sciences

When several varieties of sorghum grain varying in tannin content were fed to chicks, a high negative correlation (r = -0.92) was observed between tannic acid equivalents (TAE) of the grain and growth rate. An identical correlation (-0.92) was noted between TAE and feed efficiency (gain/feed). Supplementing a high tannin sorghum (HTS) diet with ferric chloride (0.5 or 1.0%) to enhance tannin oxidation failed to overcome the detrimental effects of tannin. Similarly, the addition of diethyldithiocarbamate (0.1 or 0.2%) to a HTS diet to inhibit polyphenol oxidase, and thus reduce tannin oxidation, did not affect the tannin toxicity.

Chicks fed HTS diets produced significantly more excreta while consuming less feed than chicks fed low tannin sorghum (LTS) diets. Hence, dry matter utilization of chicks fed HTS was significantly poorer than chicks fed LTS. The growth depression produced by feeding HTS in a sorghum-soybean meal diet could be completely overcome by methionine supplementation. However, the addition of methionine had no effect on dry matter utilization. These results suggest that the growth depression associated with feeding HTS cannot be explained on the basis of reduced digestibility. Since the reduced feed efficiency observed with HTS is not completely corrected by methionine supplementation, lower digestibility appears to be the cause of the poorer feed efficiency.

Other evidence that the detrimental effects of tannins cannot be completely explained by reduced digestibility was afforded by studies where the digestion of all dietary protein, carbohydrate and lipid was

circumvented by the use of crystalline amino acids, glucose and free fatty acids. When a methanol extract (TAE = 40%) of HTS was added to this diet at a level of 2.5%, a depression in growth rate was still obtained.

Although methionine supplementation is very effective in overcoming the growth depression of chicks fed HTS in a sorghum-soybean meal diet, it is without effect in certain other diets. In a sorghum-crystalline amino acid diet where the essential amino acids are added in the same amounts found in soybean meal and non-essential nitrogen is provided by glutamic acid, HTS reduces chick growth rate and additional methionine is ineffective in overcoming the growth depression. Similarly, the growth depression obtained by adding a methanol extract of sorghum tannins to a purified diet does not respond to methionine supplementation. Why methionine is effective in one case and not in others remains a rather perplexing question.

It has consistently been observed that feeding HTS to chicks results in leg anomalies characterized as a bending or bowing of the leg bones. This was first observed in chicks fed a HTS-soybean meal diet and the incidence of the abnormality usually ranged from 7-10%. The incidence of the condition increased dramatically (60-70%) when HTS was incorporated into a sorghum-crystalline amino acid diet. Increasing levels of vitamins and minerals failed to overcome the bone anomalies caused by HTS. Since bone ash is normal in the afflicted chicks, it is suggested that the defect is in the organic matrix of the bone. Therefore, it was hypothesized that tannins may be absorbed and affecting the bone collagen in a manner similar to their tanning effect in the processing of leather. This involves an increased cross-linking of the collagen fibers due to binding of tannins with collagen. Preliminary studies indicate that there may be an increased

cross-linkage of collagen fibers in both bone and muscle as indicated by a reduction in the amount of extractable collagen in these tissues from birds fed HTS.

E. RESEARCH PUBLICATIONS (ABSTRACTS)

ABSTRACT

DETERMINATION OF PROTEIN IN TANNIN-PROTEIN PRECIPITATES
Ann E. Hagerman and Larry G. Butler

The amount of protein precipitated by tannin under a variety of conditions is measured by using radioiodinated protein. The protein is mixed with purified tannin, finely ground plant tissue, or an unpurified plant extract; the mixture is centrifuged to remove the insoluble tannin-protein complex. An aliquot of the supernatant layer is counted to calculate the amount of protein precipitated. Complex formation is dependent on the pH and solvent composition. Tannin specific activity is useful for comparing tannin from various sources and for monitoring tannin purification. It is defined here as the ratio between the amount of protein precipitated and the amount of oxidizable material present.

J. Agriculture & Food Chemistry, 1980
Vol. 28: 944-947

ABSTRACT

CONDENSED TANNIN PURIFICATION AND CHARACTERIZATION OF TANNIN-ASSOCIATED PROTEINS
Ann E. Hagerman and Larry G. Butler

The conventional isolation method has been modified in order to minimize protein contamination of tannin purified from high tannin sorghum. The two unique steps of the new procedure are preliminary extraction of the ground grain with ethanol and treatment of the partially purified tannin with phenol to remove traces of noncovalently bound protein. Tannin-associated protein removed by phenol treatment is not a random mixture of all the seed proteins, but consists of several discrete components which have been isolated and partially characterized. These proteins are quite hydrophobic, and one is rich in proline. With only minor changes, the purification method can be used to isolate tannin from seeds of other plants such as legumes.

J. Agricultural & Food Chemistry, 1980
Vol. 28: 947-952

ABSTRACT

TANNIN CONTENT AS A FUNCTION OF GRAIN MATURITY AND DRYING CONDITIONS IN SEVERAL VARIETIES OF SORGHUM BICOLOR (L.) MOENCH
Martin L. Price, Andrew M. Stromberg, and Larry G. Butler

Twelve varieties of sorghum grain were assayed for tannin content at various stages of maturity. For varieties which were found to contain tannin, maximum values per seed were obtained between 25 and 40 days after half-anthesis. Wide variability in apparent loss of tannin as the grains matured was found between varieties, with tannin contents of mature seed ranging from 3 to 93% of the maximum found in the immature seed. We suggest that varieties with maximum decreases in tannin may be similar to low tannin varieties in the nutritional quality of the mature grain, yet provide bird resistance during immature stages. Drying immature grain at room temperature after boiling for 3 min or freezing caused a drastic reduction in apparent tannin content over untreated controls, but these treatments had little effect on nutritional quality of the grain.

J. Agricultural & Food Chemistry, 1979
Vol. 27: 1270-1274

ABSTRACT

PROTEIN PRECIPITATION METHOD FOR THE QUANTITATIVE DETERMINATION OF TANNINS
Ann E. Hagerman and Larry G. Butler

The tannin content of crude plant extracts or of purified preparations was determined by adding the sample to a standard solution of protein, isolating the insoluble tannin-protein complex, dissolving it in alkaline solution, and measuring the absorbance at 510 nm after adding ferric chloride. Plots of absorbance as a function of the amount of tannin are linear for tannic acid and partially purified sorghum tannins for amounts of tannin ranging from 0.20 to 1.0 mg. Non-tannin components of crude methanolic extracts of sorghum and cowpeas do not interfere with the assay. The results of the precipitation method are qualitatively similar to results obtained with the vanillin assay. The precipitation assay can be used to study the effects of pH and other parameters on tannin-protein interactions.

J. Agriculture & Food Chemistry, 1978
Vol. 26: 809-812

ABSTRACT

A CRITICAL EVALUATION OF THE VANILLIN REACTION AS AN ASSAY FOR TANNIN IN SORGHUM GRAIN
Martin L. Price, Steve VanScoyoc and Larry G. Butler

Several parameters of the vanillin assay were examined to determine which must be most closely controlled to ensure accuracy and reproducibility. A 20-min extraction in methanol was found to be adequate. When corrected for background color, the modified vanillin assay was found to give nearly identical values with those obtained with the regular vanillin assay, except with group II sorghum. The reactions of tannin and catechin, the usual standard, with vanillin were found to differ markedly in reaction kinetics. Assays of purified tannin showed that use of catechin equivalents over-estimates tannin content. The assay was found to be extremely temperature dependent. Revised procedures for the vanillin assay are presented which give excellent reproducibility.

J. Agriculture & Food Chemistry, 1978
Vol. 26: 1214-1218

ABSTRACT

OVERCOMING THE NUTRITIONALLY HARMFUL EFFECTS OF TANNIN IN SORGHUM GRAIN BY TREATMENT WITH INEXPENSIVE CHEMICALS
Martin L. Price, Larry G. Butler, John C. Rogler and William R. Featherston

Treatment of high-tannin sorghum grain with moist, alkaline conditons was shown to substantially reduce the amount of tannin as measured by three chemical assays. Chicks fed a high-tannin grain (Savannah III), treated as the whole grain with dilute ammonium hydroxide for 30 days, showed 3-week weight gains and feed efficiencies which were statistically equivalent to those of chicks fed an untreated low-tannin control (RS-610). A shorter treatment of a ground high-tannin grain (BR-54) with a 0.5 M aqueous solution of K_2CO_3 resulted in a comparable improvement in weight gains and a substantial improvement in feed efficiencies. Treatment of the same grain with moisture and CaO gave an improvement of a lesser magnitude. Increases in available protein after treatments did not appear sufficient to account for the nutritional improvements.

J. Agriculture & Food Chemistry, 1979
Vol. 27: 441-445

ABSTRACT

EFFECTS OF HIGH TANNIN SORGHUM, TANNIC ACID, CATECHIN AND SORGHUM TANNIN EXTRACT ON THE PERFORMANCE OF YOUNG CHICKS
J. C. Rogler, W. R. Featherston, R. G. Elkin and R. K. Giles

The inc usion of either high tannin (HT) sorghum, tannic acid or catechin in a sorghum-soybean meal diet depressed growth rate to about the same extent. Supplemental methionine completely overcame the growth reduction caused by HT sorghum, but had little influence on the detrimental effects of tannic acid or catechin. Feed conversion was adversely influenced by all 3 sources of tannin with the greatest effect observed in chicks fed HT sorghum. Additional methionine improved feed conversion of chicks fed HT sorghum or catechin, but had little effect on chicks fed tannic acid. Although HT sorghum depressed growth rate and feed conversion in a sorghum-peanut meal diet, the magnitude of the effect was much less than with a sorghum-soybean meal diet. As with the soybean meal diet, supplemental methionine completely overcame the detrimental effects of HT sorghum on growth and partially overcame the effects on feed conversion in the peanut meal diet. A methanol extract of HT sorghum containing 40% tannic acid equivalents depressed performance of chicks fed a sulfur amino acid-limiting diet where digestion of exogenous protein, carbohydrate and lipid was by-passed by the use of crystalline amino acids, glucose and fatty acids. The addition of methionine to diets with and without tannin extract improved growth rate and feed conversion by about the same percentage, but the methionine supplemented diet containing tannin extract was still inferior to a similarly supplemented diet without extract.

Proceedings of XVI World's Poultry
Congress, 1978
Vol. 2: 1094-1103

ABSTRACT

STUDIES ON THE UTILIZATION BY CHICKS OF SORGHUM GRAINS WITH VARYING TANNIN CONTENTS
W. R. Featherston, J. C. Rogler and R. K. Giles

Studies were conducted on the influence of sorghum grain tannins on weight gain, feed efficiency, dry matter utilization and nitrogen retention by young chicks. Weight gain and feed efficiency of chicks were inversely related to the tannic acid equivalents of the grains in a growth trial utilizing seven varieties of sorghum grain. Significantly ($P < 0.05$) lower dry matter utilization and nitrogen retention were observed with chicks fed high tannin as compared with low tannin sorghum grain. Supplementation of the high tannin sorghum diet with 0.15% DL-methionine or 1% polyvinylpyrrolidone had no effect on dry matter utilization but resulted in a significant ($P < 0.05$) improvement in nitrogen retention. Supplementation of the high tannin sorghum diet with methionine resulted in comparable weight gains as with chicks fed the low tannin sorghum diet similarly supplemented. The ability of methionine to overcome the growth depression noted in chicks fed the high tannin diet without improving dry matter utilization indicates that tannins are exerting their influence in some manner in addition to decreasing the digestibility of the diet.

Proceedings of XVI World's Poultry
Congress, 1978
Vol. I: 1087-1093

ABSTRACT

INVESTIGATIONS OF LEG ABNORMALITIES IN CHICKS CONSUMING HIGH TANNIN SORGHUM GRAIN DIETS

R. G. Elkin, W. R. Featherston and J. C. Rogler

Studies were conducted into the etiology of leg abnormalities noted in chicks fed high tannin sorghum grain diets. These anomalies were characterized by a bowing of the legs with a swelling of the hock joints. The incidence of these leg problems was found to be markedly higher when the amino acids provided in the diet by soybean meal were replaced by crystalline amino acids. Supplemental vitamins and minerals had no alleviating effect on the leg problem. Bone mineralization was apparently not influenced by tannins as demonstrated by similar bone ash values for chicks fed high or low tannin sorghums. A possible tannin-caused alteration in the organic matrix of bone is discussed.

High tannin sorghum depressed chick growth and feed conversion when campared with low tannin sorghum in both sorghum-soybean meal and sorghum-amino acid rations. This growth depression was overcome by supplementing the high tannin sorghum-soybean meal diet with 0.15% DL-methionine, but no growth response was observed with a similar supplementation of a high tannin sorghum-amino acid diet.

Poultry Sci., 1978
Vol. 57: 757-762

ABSTRACT

CURRENT STATUS OF PROTEIN QUALITY IMPROVEMENT IN GRAIN SORGHUM
J. D. Axtell, S. W. VanScoyoc, P. J. Christensen and G. Ejeta

The Ethiopian high-lysine gene is associated with an 80% increase in the lysine concentration of whole sorghum grain. It is generally accepted that the gene reduces yield in all genetic backgrounds. We believe that the Ethiopian high-lysine gene, in its native background genotypes, has greatest applicability as a special purpose grain for people requiring a high-protein food source, i.e. pregnant and lactating women and weaned children, as an alternative to externally supplied supplements. [Caution - Very recent studies by Graham at Johns Hopkins (personal communication to E. T. Mertz) suggest that sorghum flour is quite indigestible by infants and small children. Since this is contradictory to results with laboratory animals, more information is needed before high-lysine sorghums are utilized as weaning foods.] The chemically induced P-721 opaque gene is associated with a 60% increase in the lysine concentration of whole grain in isogenic comparison with the sib line. The lower rate of dry matter accumulation during development on a per seed basis confirms that this gene also is associated with reduced seed weight. However, selection experiments indicate that yield component compensation, particularly in seed number, allows high yielding P-721 opaque lines to be developed. The increment in lysine concentration contributed by the gene in high-yielding genetic backgrounds may not be as great as in the isogenic line.

Proceedings of a Symposium on Seed Protein Improvement in Cereals and Grain Legumes, Neuherberg, 4-8 September 1978
Vol. 2:357-365

ABSTRACT

LYSINE LEVEL IN SOLVENT FRACTIONS OF PEARL MILLET
Christian C. Nwasike, Edwin T. Mertz, Robert C. Pickett, D. V. Glover, B. A. K. Chibber, and Stephen W. VanScoyoc

A typical whole grain sample of normal pearl millet was separated into five fractions by the Landry-Moureaux method. The distribution of proteins among the five fractions resembled that found in corn and not that in sorghum. Complete amino acid analysis of the fractions was made. Of special interest was the low level of lysine in fraction V, which is similar to that found in sorghum. On the basis of this finding, it is predicted that high lysine pearl millet when identified will resemble high lysine sorghum, not opaque-2 corn, in its total lysine content.

J. Agricultural & Food Chemistry,
December, 1979
Vol. 27: 1329-1331

ABSTRACT

IN VITRO DIGESTIBILITY OF HIGH-TANNIN SORGHUM AT DIFFERENT STAGES OF DEHULLING
Bakshy A. K. Chibber, Edwin T. Mertz, and John D. Axtell

High-tannin sorghum grain was subjected to sequential dehulling to remove tannins. Stepwise removal of tannins gradually increased the percent of nitrogen solubilized by pepsin from 22 to 71% and by trypsin-chymotrypsin mixture from 3 to 35%. These studies suggest that a simple pepsin digestion could be used to predict the biological value of high-tannin sorghums.

J. Agriculture & Food Chemistry, 1980
Vol. 28: 160-161

F. THESIS RESEARCH ABSTRACTS

ABSTRACT

GENETIC VARIABILITY IN A BROAD-BASED SORGHUM POPULATION
Thomas Bittinger

Genetic variability was investigated in the diverse, random-mating grain sorghum population, PP9. A Design I mating system was used where 50 random pollen parents were each crossed to a different set of three random seed parents. The 150 resulting progenies were evaluated in a randomized complete-block design over two years at the Purdue Agronomy Farm.

The characters evaluated were days to 1/2 bloom, height, lodging, panicle weight, seed weight, panicle length, and grain yield.

Analysis of variance was performed for each trait, and estimate of the additive genetic variance and the dominance genetic variance were made. Additive genetic variance was greater than dominance variance for all traits except yield. The ratio of dominance variance to additive variance was 1.24 for yield and between 0.18 and 0.66 for height, lodging, panicle weight, and seed weight. Negative dominance estimates were obtained for days to 1/2 bloom and panicle length.

Phenotypic and genetic correlations between the traits were calculated. Yield showed highly significant positive correlations with days to 1/2 bloom and panicle weight. Other significant positive correlations included height with days to 1/2 bloom, height with lodging, panicle weight with days to 1/2 bloom, and panicle weight with lodging.

Expected genetic gains were calculated for various selection procedures and heritabilities were presented.

The effects of major height and maturity genes on variation for yield was discussed, and separate estimates of genetic variances were calculated for yield after adjustment was made for maturity.

Ph.D. Thesis, 1979
R. P. Cantrell, Professor

ABSTRACT

SELECTION FOR YIELD AND LYSINE CONCENTRATION AMONG OPAQUE P-721 DERIVED GRAIN SORGHUM LINES
Paul Christensen

The expected results of multitrait index selection for yield (YLD) and lysine concentration of sorghum grain, as estimated by acid orange 12, Udy, dye-binding capacity (DBC), are evaluated for a population of F_4 derived lines, homozygous for the P-721 high lysine opaque gene. The lines were tested in the F_5. The objective for selection is overall improvement of selections from unreplicated nurseries. Optimum selection indexes are evaluated under 4 alternative sorghum grain valuation functions. All value functions give a relative value of 1.0 for normal sorghum, and each gives a relative value of 3.0 at 2 to 4 times normal sorghum DBC, or lysine concentration. The value functions are of the form

$$T = a_{YLD}(YLD) + A_{DBC}(YLD)\ (DBC-u),$$

where a_{YLD} and A_{DBC} are the relative values for production of nonlysine and lysine components of grain respectively, and where u is a constant.

The first-order approximation to the quadratic merit is good in the relevant value range. Quadratic index theory contributes to the specification of appropriate approximate first-order values for the measured traits.

Where lysine production received its lowest relative value (T_1 = 3.0 when DBC = 4 times the normal DBC for sorghum), the optimal index is associated with a decline in expected DBC and an increase in expected yield. Where lysine production received its highest relative value (T_4 = 3.0 when DBC = 2 times normal), the optimal index is associated with increases in both DBC and yield. By using relative trait values obtained

form the first-order approximation to the quadratic merit function as index weights one reduces expected gains by less than 10% compared to the optimum index. These relative trait values are good selection index weights.

The reduction in expected gain when protein is removed from the index is only about 2%. Measurement of protein concentration is not necessary in nursery selection. The covariances of protein with DBC and with yield did help specify the best selection index when DBC is relatively valuable. Optimum selection indexes were associated with reduction in the expected protein concentration. By using restricted selection index theory one can determine indexes which are not expected to reduce protein concentration. Those indexes are more likely to increase DBC.

Ph.D. Thesis, 1978
J. D. Axtell, Professor

ABSTRACT

SELECTION FOR GENETIC MODIFIERS THAT IMPROVE THE OPAQUE KERNEL PHENOTYPE OF P-721, HIGH LYSINE SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH)
Gebisa Ejeta

Vitreous endosperm, high lysine sorghum lines were developed by crossing P-721 opaque with plants in diverse sources of sorghum populations, and by mutation induction using diethyl sulfate (DES).

Approximately 12,000 F_6 heads (from crosses of P-721 opaque with sorghum plants of diverse genetic background) were screened over the light box for vitreous endosperm kernels. A total of 120 heads segregating for vitreous kernel types were selected as possible kernel modifications influenced by so-called modifier genes of the P-721 opaque locus. Further evaluations of opaque and vitreous kernels from these 120 "modified" types resulted in only 15 elite selections with stability in vitreous endosperm phenotype and lysine concentration. There was strong evidence supporting genetic background differences among the family groups evaluated.

Modified endosperm selections were consistently higher in kernel weight and grain yield, and lower in both percent protein and lysine concentration. Total protein yields and total lysine yields of the modified endosperm selections were only 1 percent and 4 percent lower, respectively, than that of their opaque checks.

Kernel weight increased with increase in the degree of kernel modification, with the most vitreous types showing the highest test weight. The trend in percent protein and lysine concentration was in favor of the opaques and the less modified kernel types.

Treatment of seeds of P-721 opaque, high lysine sorghum line with

diethyl sulfate (DES) also resulted in mutants with vitreous endosperm and high lysine concentration. After five generations of selection, 23 vitreous endosperm, high lysine mutants were identified. Of these, mutants 5589, 5458, and 0226 were found to be stable across generations in kernel phenotype and lysine concentration. On the other hand, a large number of vitreous endosperm mutants showed lysine concentration equivalent to normal sorghums. The reversion to wild-type phenotype (vitreous endosperm and low lysine concentration) of these mutants is speculated to be not in the true reversal of the primary genetic event (a rare occurrence) but possibly in the introduction of an additional mutation somewhere else in the genome masking the expression of the primary mutation, P-721 opaque. These putative suppressor mutants need to be confirmed, however.

In the DES-induced population of mutants, a family of mutant plants with heritable red leaf characteristics was identified. Seeds from the red leaf mutant (5279) plants contain a range in protein concentration varying from 20-28 percent of sample. This red leaf character was found to be due to a single recessive gene.

Analysis of kernels from normal (green leaf) and red leaf mutant plants segregating in an F_2 generation of the cross (PP14(ms_3) x 5279) showed that this mutant is responsible for the high protein concentration in the grain. Two possible explanations for the genetic mechanism involved in the increased concentration of grains from red leaf mutant, 5279, are described.

Both mutation induction and pedigree breeding procedures utilized in this study were effective in generating vitreous endosperm, high lysine sorghum lines. However, the frequency of useful mutants generated from

mutation induction was lower. In addition, the mutation induction approach was more expensive and demanded large number of progenies be evaluated for successful identification of mutants.

Ph. D. Thesis, 1979
J. D. Axtell, Professor

ABSTRACT

EVALUATION OF LEAF ANTHRACNOSE, HEAD BLIGHT AND RED STALK ROT OF SORGHUM CAUSED BY COLLETOTRICHUM GRAMINICOLA
Alexandra de Silva Ferreira

Of 23 sorghum lines evaluated for resistance to three isolates of Colletotrichum graminicola (Ces.) Wils., nine were susceptible, six moderately resistant, and eight resistant to the leaf blight phase. Some of these lines demonstrated resistance to the head blight phase. The leaf blight phase was more destructive than either the red stalk rot or head blight phases.

No complete resistance to the red stalk rot phase of anthracnose was found, but genotypes varied in the amount of discoloration observed in the pith after inoculation. Resistance to red stalk rot appeared to be independent of the leaf and head blight phases. The stalks of sorghum were not severely rotted and no lodging was observed in the inoculation treatments.

Genotypes varied in their reaction to head inoculation and genotypes could be classified as resistance or susceptible.

Yield in susceptible sorghum genotypes was significantly reduced by leaf blight infection when compared to the non-inoculated control, but yields were not significantly reduced by either the red stalk rot or head blight infections. Yield losses also varied according to genotypes with the head and red stalk rot phases. However, a resistant genotype such as 954206 would be preferable to the highly susceptible genotype 954114.

Sorghum genotypes can be screened for resistance to leaf anthracnose in the greenhouse when plants are inoculated 35 days after planting. The greenhouse inoculations on sorghum lines were highly correlated (r = 0.87)

with field inoculations (49 days after planting). The disease was usually more severe in the field than greenhouse. No leaf infection was observed when sorghum plants were inoculated 15 days after planting and a few lesions were found on susceptible genotypes 25 days after planting.

These results indicate that none of the methods are suitable for determining resistance to all three phases of anthracnose, but the leaf blight method is more reliable and can also be used to assay for head blight resistance.

M.S. Thesis, 1979
H. L. Warren, Professor

ABSTRACT

SORGHUM TANNINS: INHERITANCE, SEASONAL DEVELOPMENT AND BIOLOGICAL VALUE
Rebecca Hartigan

Sorghum can be separated into three groups according to tannin properties in the seed. Group I sorghum has no tannin and, therefore, no pigmented testa. Group II sorghum grain has tannin extractable only with acidic methanol; group III sorghums contain tannins extractable with absolute methanol as well as acidic methanol. Group II and group III sorghums have a pigmented testa. Group III sorghum tannin developed in the testa throughout the season and remained at maturity. When fed to rats, the group III sorghums significantly reduced weight gain and feed efficiency. Group II sorghums had methanol-extractable and acidic methanol-extractable tannins in the immature seed. The concentration of these tannins was as high in one developing group II variety as in the group II sorghums, but the methanol-extractable tannin disappeared completely by maturity. When fed to rats, group II sorghums gave weight gains and feed efficiencies comparable to the group I sorghums.

Two epistatic genes TW and TH controlled tannin characteristics in crosses between group II and group I sorghums. TW and th produce group II sorghums, tw and TH produce group III sorghums. TW is epistatic over TH and th is epistatic over tw. Additional genes for tannin groupings were indicated in crosses between group III and group I sorghums, but their mode of action is not known.

M.S. Thesis, 1979
J. D. Axtell, Professor

ABSTRACT

THE INHERITANCE OF RESISTANCE TO *COLLETOTRICHUM GRAMINICOLA* IN GRAIN SORGHUM, *SORGHUM BICOLOR*
Ellen Margaret Jones

This study was designed to determine the inheritance of grain sorghum leaf, panicle and grain resistance to the fungal pathogen, *Colletotrichum graminicola*. Four grain sorghum lines were used in this study: 954206 (R-1), IS 9569 (R-2), 954114 (S-1), and IS 4225 (S-2). All possible resistant by susceptible crosses were made; also the two resistant parents were crossed. The parent R-2 was selected in order to determine if the resistance in this late-maturing line had a genetic component. Leaves of all plants in parent, F_1, F_2, F_3 and the first backcross generation (BC_1) were artificially inoculated with a conidial suspension of the pathogen and plants were later given a disease severity rating on leaf, panicle and grain. Segregation patterns from progeny generations were used to postulate genetic models.

Leaf resistance was shown to be controlled by a single completely dominant gene in R-1, while at least two completely dominant, independent genes were present in R-2. No reciprocal differences were noted for the progeny of any cross. It was found that the genetic background of each parent apparently modified the level of leaf susceptibility of its progeny.

Progeny data for panicle and grain disease ratings did not indicate consistent support for any genetic model. A limited quantity of inoculum from leaf lesions as well as unfavorable weather conditions reduced the reliability of disease ratings. However, it was concluded that resistance to panicle and grain infection was dominant to susceptibility, and that relatively few major genes were involved in control of resistance. No

reciprocal differences were found in progeny data.

Correlation coefficients between diseased parts of F_2 plants were computed. Positive correlations were found between panicle and grain, and between leaf and panicle, but no correlation was found between leaf and grain.

The results of this study indicated that leaf resistance is controlled by relatively few major genes, and therefore, that leaf resistance can be efficiently transferred to other germplasm in a sorghum breeding program. The discovery of at least two different sources of resistance in R-2 offers further breeding opportunities for the incorporation of resistance to *C. graminicola*.

Ph.D. Thesis,
J. D. Axtell, Professor

ABSTRACT

ROOT AMMONIUM ASSIMILATION ENZYME ACTIVITIES, GRAIN STORAGE PROTEIN AND GRAIN YIELD RESPONSE TO HIGH AMMONIUM FERTILIZER AMONG MAIZE HYBRIDS
Tim Clifford Murphy

A whole plant hypothesis linking genotypic differences in efficiency of ammonium assimilation and utilization to grain yield under high ammonium fertilizer levels was investigated in genetically diverse maize single cross hybrids. Four hybrids and 5 parental inbreds were grown in the growth chamber and sampled at the 3-leaf stage. Comparisons were made between hybrids and inbreds and within each group for root ammonium assimilating enzyme activity levels, root and shoot dry weights, nitrogen contents, and root protein contents with zero ammonium and 25 mM ammonium treatments.

Glutamate synthase (GOGAT) activity per gram root tissue was significantly higher in inbreds than hybrids in response to 25 mM ammonium but total GOGAT per plant did not differ significantly between inbreds and hybrids.

Glutamate dehydrogenase (GDH) specific activity and activity per plant was significantly higher in hybrids than inbreds as was root and shoot total weight, nitrogen content, and root total protein. Root and shoot nitrogen percents were significantly higher in inbreds.

Glutamine synthetase (GS) did not differ significantly on a per protein or per root tissue basis between hybrids and inbreds. All three enzymes showed increased levels of activity in response to 25 mM ammonium.

Among inbreds response to 25 mM ammonium indicated the root nitrogen percent and root free nitrogen percent correlated significantly negatively with GOGAT specific activity, activity per root tissue and activity per

plant. GDH specific activity and activity per root tissue correlated significantly positively with seedling shoot growth in inbreds.

Among hybrids response to 25 mM ammonium indicated both GOGAT and GDH specific activities correlated highly negatively with shoot total weight at the three leaf stage. Root nitrogen percent correlated significantly negatively with GS specific activity and root growth.

Grain yield in 2 years field trials among the 4 hybrids correlated highly significantly with GOGAT specific activity, activity per gram root tissue and activity per plant in growth chamber seedlings. Grain yield correlated significantly with GDH specific activity and highly positively with GS per gram root tissue. Grain yield also correlated highly significantly negatively with shoot total nitrogen of seedlings. All three activities, GOGAT, GDH, and GS on a per root tissue basis correlated significantly with grain total protein content and highly positively with grain total zein. Grain yield and protein contents were correlated highly negatively with root free nitrogen percent. These results suggest that genotypic ammonium assimilation enzyme differences in activities accentuated under high ammonium stress may correlate with grain yield of genetically elite maize hybrids grown with high ammonium fertilizer in a nearly optimal environment.

Genotypic differences in grain yield and grain total protein, albumin plus globulin, and zein protein fractions among 12 genetically diverse maize single cross hybrids were investigated as sinks for grain dry matter and assimilated organic nitrogen in each of 2 years' yield trials. Yield did not correlate consistently with kernel weight, kernel number, or any of the protein fraction contents among genotypes. However, significant kernel weight differences from the base to the tip of the ears of 12 single cross

hybrids overall did correspond to parallel significant differences in zein storage protein percents. Non-significant differences in non-zein storage percents were found. These results suggest that utilization of the assimilated nitrogen as storage proteins in the grain does not correlate with grain yield among genotypes although the importance of this effect on yield may be confounded with genotypic whole plant differences affecting supply and rate of supply of assimilated carbon and nitrogen to the grain sinks.

Ph.D. Thesis, 1980
J. D. Axtell, Professor

ABSTRACT

MODIFICATION OF THE OPAQUE ENDOSPERM PHENOTYPE OF THE HIGH LYSINE SORGHUM LINE P-721 (SORGHUM BICOLOR (L.) MOENCH), USING THE CHEMICAL MUTAGEN DIETHYL SULFATE
Kay Scott Porter

Acceptance of high lysine sorghum (Sorghum bicolor (L.) Moench) by both growers and consumers has been limited by problems associated with the opaque endosperm of high lysine lines. In this study, vitreous high lysine mutants of sorghum were induced by treating seeds of an opaque high lysine line, P-721, with the chemical mutagen dithyl sulfate (DES). Putative mutants were identified by screening M_2 seeds from individual self-pollinated M_1 heads over a light box. Vitreous segregates in otherwise opaque heads were considered to be possible modified types and were subsequently evaluated for protein quality and degree of vitreousness.

Putative mutants were grown in head rows in the M_3 and M_4 generations and self-pollinated heads were harvested. Individual heads in each row were kept separate at each generation. Non-viable mutants or "mutants" classified as opaque in the M_3 generation were discarded.

Only the most vitreous mutants in each generation were evaluated chemically. The remaining (less vitreous) ones were carried to subsequent generations for analysis. Possible mutants were evaluated for either percent protein and lysine (as percent of protein and percent of sample), or dye-binding capacity (DBC) and percent protein. Vitreous segregates at the M_2 and M_3 generations were compared to opaque samples from the same heads. Mutants at M_4 were compared to opaque P-721 and its normal-lysine, non-opaque sib.

Three hundred and thirty-five vitreous endosperm mutants which were similar to their opaque checks in protein quality were identified on the

basis of initial chemical evaluations. Aproximately 70% of the mutants evaluated initially in either the M_2, M_3 or M_4 generations had lysine as percent of protein greater than 2.5%.

Mutants were evaluated in subsequent generations to assess the stability of both protein quality and modified endosperm phenotype. Fifty-six mutants were superior in protein quality (lysine greater than 2.5% or DBC greater than 40) in at least two generations. Thirteen of the 56 mutants were homozygous vitreous at M_4 and were selected. Lysine content (percent of protein) of the selected mutants averaged 2.65% at M_3 and 2.61% at M_4. Lysine as percent of sample averaged 0.36 in M_3, but reduced protein in all mutants at M_4 resulted in slightly lower values (0.29) in that generation.

Mutants varied considerably in the degree of vitreousness in the endosperm. Kernel maturity (influenced by the duration of the growing season) seemed to be the factor most important in altering the degree of vitreousness. Immature kernels were always less vitreous than fully mature kernels which may have resulted in the discarding of later maturing types.

Most modified mutants with protein quality approaching that of their opaque counterparts were intermediate in vitreousness. A high degree of vitreousness was most often associated with relatively poorer protein quality although exceptions were found. Of the 13 homozygous vitreous mutants selected, two were similar in appearance to the non-opaque, normal sib of P-721. Seven mutants were intermediate in the degree of vitreousness and four were only slightly modified. Mutants having slightly poorer protein quality but with more vitreous phenotypes were also selected.

Mutation induction was effective in generating a large number of

mutants in an opaque, high lysine background. Mutants approaching opaque in protein quality (lysine content) but with modified, vitreous endosperms, were selected for use in conventional breeding programs. Such mutants may prove useful in overcoming some of the problems associated with the soft opaque kernel phenotypes, and may improve grower and consumer acceptance of grain sorghums with high protein quality.

Ph.D. Thesis, 1977
J. D. Axtell, Professor

ABSTRACT

VARIATION FOR STALK CHARACTERISTICS AND THEIR ASSOCIATION WITH LODGING AND HEAD WEIGHT IN A DIVERSE SORGHUM POPULATION.
Thomas Prest

Variation for stalk characteristics and their association with lodging and head weight were analyzed in a diverse sorghum population, PP9, using 80 S_2 lines. These lines were categorized into three height groups, the tall (greater than 170 cm), medium (110-170 cm), and short (less than 110 cm). The stalk characteristics studied included, rind thickness, rind puncture, third internode density, third internode diameter, and stalk juiciness. The stalk measurements were taken at physiological maturity.

Significant differences between lines within height groups were observed for internode diameter, density, and rind thickness. Differences in rind puncture between lines in the medium and short groups were significant in 1977 but not in 1978, whereas differences in rind puncture in the tall group were significant in 1978 but not in 1977.

Significant differences in lower stalk breakage were observed between lines within the medium and tall groups. Such breakage was negligible in the short group. However, differences in peduncle breakage were nearly significant.

Third internode diameter, rind thickness, and third internode density had significant negative correlations with lower stalk breakage in the tall group. Within the same group, rind puncture was not significantly correlated with stalk breakage.

Third internode diameter and rind thickness showed significant negative correlations with lodging in the medium group. Rind puncture and

internode density were not significantly correlated with lodging.

None of the characteristics of the lower internodes were significantly correlated with peduncle breakage in the short group.

The correlations were generally low in all groups. Thus, the given stalk measurements, when taken at physiological maturity, should not be considered as equal replacements for field estimates taken later on in the season. However, selection for increased diameter and/or increased rind thickness may still be effective in increasing lodging resistance.

Lower stalk breakage was not significantly correlated with head weight in either the tall or medium groups. Correlations between head weight and the stalk characteristics under study were generally not significant. Pertaining to PP9, these data suggest that recurrent selection for increased yield without selection for lodging resistance may not decrease lodging resistance in the selected populations. Concurrent selection for both traits is still advised.

M.S. Thesis, 1979
R. P. Cantrell, Professor

ABSTRACT

THE EFFECT OF TANNINS ON NUTRITIONAL QUALITY OF DRY BEANS, *PHASEOLUS VULGARIS* L.
Richard Ronnenkamp

Tannin compounds are found in many foods and often are associated with a bitter taste. In sorghum, tannins are a factor in the nutritional quality of the grains. Dry beans have toxic factors that influence the nutritional value, but tannins are not included.

From the dry bean collection, 69 entries were selected from a variety of locations and seed color. The entries were analyzed for tannin content. A white, black, and red bean were selectd for low, medium, and high tannin content. These beans were used to prepare eight diets for evaluation with weanling rats. The testae were removed or extra testae were added to change the tannin content of the diets. The influence of tannins on the albumin and globulin portion of the protein for the beans used in the diets and for other sources of white, black, and red beans was determined. The association of tannin content with color was observed in the F_2 generation of crosses of white and red beans.

A range of tannin content was found in the entries. White beans were zero and colored beans ranged from very low to 2.5 catechin equivalents. the highest values were found in red beans. The weight gain and protein efficiency ratio of rats was improved when the red testa of the red bean was replaced with a white testa and the tannin content was reduced from 2.5 CE to almost zero. The weight gain and protein efficiency ratio of rats decreased as the tannin content of the beans in the diests increased.

The tannin content did not influence the distribution of the albumins and globulin fractions of the bean protein, but changes in these fractions

were observed between bean types.

The use of initial rat weight as a covariate in analyzing the data was significant for the dependent variables weight gain and food consumption, but not for the protein efficiency ratio.

The F_2 segregation of tannin content was not controlled by the genes for testa color. A range of high and low values was observed within a given color.

Ph.D. Thesis, 1977
J. R. Wilcox, Professor

ABSTRACT

INFLUENCE OF SEED PROTECTANT ON THE TOLERANCE OF GRAIN SORGHUM TO ALACHLOR AND RELATED COMPOUNDS
Joao B. da Silva

The advent of herbicidal antidotes has opened a new field of research and created possibilities for the use of herbicides in grain sorghum (Sorghum bicolor (L.) Moench) that otherwise would be unsafe for the crop. The influence of the seed treatment with 1,8-naphthalic anhydride on the tolerance of three sorghum hybrids, RS-610, B-35 and NK-180, to three triazine herbicides, EPTC, either alone or in combination with the herbicidal antidote R-25788 (N,N-dially-2,2-dichloroacetamide), alachlor, metolachlor, propachlor and four experimental chloroacetamide herbicides, was studied in nine field experiments carried out in the 1975-1977 period, in three different locations of the state of Indiana. The influence of other factors such as soil type, soil temperature, sources and means of protection, herbicidal rates and seed protectant rates on the tolerance of the crop to alachlor was studied in four greenhouse experiments.

Results obtained from the field experiments allowed many important observations concerning the use of 1,8-naphthalic anhydride as a seed treatment to protect grain sorghum:

1. Independently of the seed treatment, damage to grain sorghum caused by alachlor varied with the rate employed, and means of application, the soil type and among sorghum hybrids. When grown on the Chalmers Silty Clay Loam, RS-610 showed more tolerance to alachlor than both B-35 and NK-180.

2. Metolachlor and RE-19790 [3-chloroacetyl-4,4-cyclohexane-spiro-(2,2-dimethyl-1,3-oxazolidine)] were

the most promising herbicides among the new chloroacetamides in both weed control and selectivity to grain sorghum. GCP-5544 (N-benzyl-N-isopropyltrimethyl acetamide) was the most selective to the crop but it failed to control weeds expecially redroot pigweed (Amaranthus retroflexus L.).

3. Atrazine and Cyanazine, applied alone or in combination with propachlor did not cause any significant injury to grain sorghum and they were unaffected by the seed treatment.

4. R-25788 (N,N-dially-2,2-dichloroacetamide) did not give any significant protection to grain sorghum when applied in tank mixture with EPTC. This herbicide was always injurious to the crop and its damaging action was partially hindered by the seed treatment with naphthalic anhydride. Although the protective action of the seed treatment was statistically significant, it was not great enough to give sorghum plants conditions to produce as much as the handweed plots in many cases.

5. As a general rule the seed treatment with naphthalic anhydride was beneficial any time its protective action was necessary. This was especially true for the chloroacetamide herbicides which were not injurious to grain sorghum all the times. When its protective action was not required, the effect of the seed treatment was unapparent or prejudicial. This negative action of naphthalic anhydride was noticed at least in three different cases, all three recorded on the Bedford Silt Loam.

Experiments carried out in the greenhouse were complementary to the field experiments and corroborated field observations. The trials provided information on the influence of soil temperature which was found to affect

the herbicide but not the seed treatment with naphthalic anhydride, and they also showed that sorghum protection from alachlor can be obtained from both naphthalic anhydride and R-25788 but only as a seed treatment.

Ph.D. Thesis, 1977
J. L. Williams, Jr., Professor

ABSTRACT

AGRONOMIC AND BIOCHEMICAL EVALUATION OF THE SORGHUM (SORGHUM BICOLOR (L.) MOENCH) HIGH-LYSINE P-721 OPAQUE GENE IN ISOGENIC AND DIVERSE GENETIC BACKGROUNDS
Stephen VanScoyoc

Yield and accumulation of panicle seed weight, number of seeds, seed weight, volume, and density, and black layer formation were compared during development of endosperm mutant P-721 (P-721-O), its normal sib line (P-721-N) and hybrids RS671 and NK300 grown at 4 populations. Performance under a stress environment was poorer and yield was reduced 15-20% in P-721-O compared with P-721-N. Yield reduction was attributed largely to smaller seed weight and density. Increased population acted as a stress environment and resulted in decreases in seed weight per panicle, seed numbers and panicles per plants for lines and hybrids. One hundred seed volume, weight and density were unaffected by increased population. Completion of black layer formation in all seeds coincided with physiological maturity, as indicated by maximum seed weight per panicle, in P-721-N at the lowest population but not in P-721-O. At high populations, date of completion of black layer was not a good predictor of maximum seed weight accumulation for any of the lines or hybrids.

F_6 opaque endosperm lines derived from crosses of P-721-O with vitreous lines, hybrids and populations and selected for yield, dye binding capacity, lodging and disease resistance as well as food grain quality were compared in a one year trial with 12 high-yielding, widely adapted vitreous endosperm controls. Eighteen opaque lines had yields equivalent to the vitreous controls (above 7000 kg/ha dry weight) while containing, on the average, one percent more protein and 0.5% more lysine as a percent of

protein. Improvement in yield of the best P-721-0 derived opaque lines was due to increases in all yield components and not to height.

Changes in seed weight, protein, oil, and amino acid profiles, and the proportions, amino acid patterns and electrophoretic mobility of Landry-Moureaux protein fractions of developing endosperms of P-721-0 and P-721-N grown at a low population were followed at weekly intervals from 10-59 days after half-bloom (DAHB).

Patterns of seed weight, volume and nitrogen content were similar to those of accumulation of seed weight per panicle. Levels in both lines increased steadily from 10 to 31 days, at which time rates were reduced for P-721-0 but not P-721-N. By 38 DAHB, accumulation had ceased in P-721-0 but continued through 45 DAHB for P-721-N. Whole seed protein content did not change during development but P-721-0 endosperm protein increased 1.2% while that of P-721-N fell 0.7%.

Landry-Moureaux fraction I albumins and globulins differed little among lines. Fraction II plus VII total kafirin accumulation plateaued at 24 DAHB for P-721-0 but continued to increase rapidly through 38 DAHB for P-721-N. Fraction IV plus V total glutelin synthesis ceased beyond 24 DAHB for P-721-N but increased steadily through 45 DAHB for P-721-0 when expressed either on a percent of total protein or on a N per endosperm basis.

Shifts in Landry-Moureaux protein fraction amino acid profiles during endosperm development were generally small and did not differ greatly among lines. Lysine content was highest in fraction I, intermediate in fractions IV and V and lowest in fraction II and III.

SDS-PAGE of endosperm protein fractions during development indicated few or no differences in banding patterns for any of the fractions and only

occasional differences in staining intensities between line bands. Ten DAHB whole seed patterns were, however, very different from those later times in development for all fractions. Kafirin bands were absent at 10 DAHB.

Optimal conditions for a rapid fractionation technique for sorghum endosperms were established. This method would be useful in a screening program for mutants with high lysine content of glutelin.

Ph.D. Thesis, 1979
J.D. Axtell, Professor

ABSTRACT

RELATIONSHIP OF TANNIN AND SEED COLOR TO SEEDLING VIGOR IN *SORGHUM BICOLOR* (L.) MOENCH
Billy Woodruff

A 2-year study was conducted to determine the relationship of tannin and seed color with germination and emergence. Fifty-seven lines were selected from the World Sorghum Collection based on tannin content and seed color. Seedling emergency under field and laboratory conditions was used as a measurement of seedling vigor. Warm germination percentages of high tannin and dark brown-seeded lines of seed harvested in 1975 were equal to seed harvested in 1977. Germination of 1977 seed showed no difference between tannin or color groups. High tannin lines of 1975 seed had a significantly higher emergence than low tannin lines. High and low tannin lines of 1977 seed were not significantly different in seedling vigor but medium tannin lines had a significantly lower vigor. Dark brown seeds were higher in emergence than light brown or nonbrown seeds under cool, wet conditions. Significant differences were observed among lines within groups for seedling vigor. Based on this data, the cold testing of dark brown-seeded lines might serve as a starting point in a breeding program interested in screening a large number of lines for increased seedling vigor.

M. S. Thesis, 1978
R. P. Cantrell, Professor

ABSTRACT

INHERITANCE OF TANNIN QUANTITY IN SORGHUM
Billy Joe Woodruff

The vanillin assay was used to estimate the tannin content of 13 F_2 populations. The analysis revealed an intermediate to high broad-sense heritability for tannin quantity. Similar F_2 segregation patterns and F_1's in many crosses suggested a few genes. High tannin was found to be dominant to low tannin. A very high tannin line indicated the presence of at least one partially dominant gene. Classification of the F_2 based on the parental and F_1 ranges of catechin equivalents suggested one or two genes were segregating in most crosses, although different gene loci or alleles appeared to exist. Fitting the expected distribution within each phenotypic class to the observed F_2 distributions indicated one or two gene models were inadequate to explain the data.

Ph.D. Thesis,1980
R. P. Cantrell, Professor

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