TRANSFER OF HIGH LYSINE TRAIT TO ADAPTED SORGHUM VARIETIES

by

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INTRODUCTION

Grain Sorghum (Sorghum bicolor, L. Moench) is used in the United States mainly for livestock feed, but around the world grain sorghum is a very important food crop for humans. Grain sorghum can be grown on more arid land and requires less fertilizer than corn. With the food shortage in the world and with the new land that is marginal for other crops being cultivated, grain sorghum will become even more important.

Studies in the past have emphasized increasing the yield and disease resistance of grain sorghum. More recently, emphasis has been placed on increasing the nutritional value of grain sorghum by increasing the quality of protein. Lysine is the limiting amino acid of grain sorghum protein and research has centered on increasing this amino acid.

A grain sorghum introduction (PI400226) was found to be high in lysine in comparision to other adapted grain sorghums. This introduction, however, has other agronomic traits that are undesirable. This present study was directed towards transferring the high lysine trait from grain sorghum introduction (PI400226) to adapted grain sorghum varieties.

REVIEW OF LITERATURE

Lysine, the limiting amino acid in most grains, is more deficient in grain sorghum (Sorghum bicolor L. Moench.) than in other major cereals (Steering Committee, 1975). Corn (Zea mays L.) also is low in lysine; however, since the discovery of high lysine in the endosperm of opaque-2 corn (Mertz et al., 1964) considerable attention has been given to the effect of this gene on the synthesis of storage proteins. The major effect of this gene in corn is to reduce the amount of zein (Mertz et al., 1964). Protein bodies are the major storage site of zein, or alcohol soluble proteins, which are extremely low in basic amino acid content, including lysine (Christianson et al., 1969). Examination of the relative number and size of the protein bodies in the corn endosperm by the scanning electron microscope has been used to distinguish high lysine corn varieties (Wassom and Hoseney, 1973).

Protein bodies of grain sorghum are similar to those of corn. They contain mainly alcohol soluble proteins, kafrine, which are very low in lysine (Virupaksha and Sastry, 1968; Sechinger and Wolf, 1973; Sullins and Rooney, 1974). The relationship between lysine and kafrine causes seeds that have numerous protein bodies to be low in lysine per protein percentage and the opposite for seeds that have few protein bodies (Hoseney, Davis, and Harbers, 1974; Virupaksha and Sastry, 1968). Examination of the number and size of the protein bodies in grain sorghum endosperm by the scanning electron microscope to distinguish between high lysine and normal varieties has also been reported (Hoseney, Davis, and Harber, 1974; Sullins, Rooney, and Rosenow, 1975).

Four grain sorghum lines have been discovered to contain higher

than normal grain lysine concentrations to date. Two Ethiopian grain sorghum lines (IS11167 and IS11758) were discovered after screening over 9,000 lines in the world sorghum collection (Singh and Axtell, 1973). Another high lysine sorghum line was discovered by Rosenow (cited in Sullins, Rooney, and Rosenow, 1975). The fourth line (P-721) is a mutant that was obtained by applying diethyl sulfate, a chemical mutagen. It was selected from 23,000 M_3 heads by placing a light box over each head and examining for segregating opaque kernals (Axtell, 1976).

Seeds of the first three lines cited were shrunken or dented and had soft endosperms, high protein concentrations, and high germ-to-endosperm ratios that might cause part of the high lysine content (Singh and Axtell, 1973; Sullins et al., 1975). The fourth line (P-721) also had a shrunken or dented seed, soft endosperm, and a high protein concentration which may cause its high lysine content (Axtell, 1976). An apparent reduction in number and size of endosperm protein bodies was noted on the two Ethiopian lines and the line found by Sullins et al. (1975). However, other observers reported finding a large amount of protein bodies in the two Ethiopian lines (Hoseney and Paulsen, 1976).

A grain sorghum introduction 'Dwarf White Milo' (PI400226) from the Republic of the Sudan was observed to contain few endosperm protein bodies under the scanning electron microscope in 1973. Lysine content of the grain measured by amino acid analysis was 3.01 g per 100g protein, significantly higher than normal sorghum grain (Hoseney et al., 1974). In contrast to the four previously discovered high lysine sorghums, Dwarf White Milo grain had normal appearing grain, normal protein concentration, and hard endosperm.

There are many techniques to distinguish high lysine seeds. Already

mentioned are the scanning electron microscope technique for corn and grain sorghum to observe number and size of protein bodies in endosperm. This technique is only qualitative and relatively expensive. There are methods that quantitatively estimate the lysine content of total seed proteins by colorimetry. Several such techniques utilize the reagents used by protein chemists to identify the N-terminal amino acid of a polypeptide chain. Since such reagents are also capable of reacting with the E-amino group of lysine; an example (Tsai et al., 1972) is 2-chloro-3, 5-dinitropyridine. Kakade and Liener (1969) used 2,4,6-trinitrobenzenesulfonic acid (TNBS) to determine the available lysine of foodstuffs. Selim (1965) used Sangers reagent. All of these methods are time consuming because of the preparation involved.

Mertz et al. (1973) reported a technique to use ninhydrin as a reagent to distinguish between high lysine and normal lysine grain. Ninhydrin reacts with free amino acids to give a color reaction. It has been reported that opaque-2 corn has higher free amino acid content than normal corn (Sodek and Wilson, 1971). Mertz obtained similar results with his ninhydrin technique on high lysine sorghum. Amino acid analysis confirmed his results.

Beckwith et al. (1975) reported a modification of the ninhydrin procedure in which he estimated lysine content in the protein portion of the endosperm by first seperating the free amino acids.

MATERIALS AND METHODS

Dwarf White Milo (DWM) grain sorghum was evaluated for general agronomic characteristics, height, and maturity at Manhattan, Kansas, during summer 1973. The introduction was crossed with three adapted varieties of grain sorghum; 'Combine Kafir 60' (C), 'Plainsman' (P), and 'Redlan' (R) during summer of 1974 at the Agronomy Farm, Manhattan, Kansas. The crosses were made by A. J. Casady. The F_1 plants were grown in the greenhouse and selfed during the winter of 1974-75. 42 (C X DWM), 39 (P X DWM), and 12 (R X DWM) F_1 plants were selected for suitable maturity among the three crosses. The F_2 seed from the 93 F_1 plants was treated with Captan and planted with a Planet Jr. on May 21, 1975. The plot area contained 13 75-cm rows with 9 10-m tiers within a row. The 12 (R X DWM) each comprised 3 10-m sections and the (C X DWM) and (P X DWM) comprised the other 81 10-m tiers. The F_1 plants were transplanted from the greenhouse to the same location. Bags were placed on 2740 F_2 plants at heading to insure selfing. Plant height (from ground to top of head) and heading date (date when head just emerged from flag leaf) were recorded. The 2740 heads were harvested individually, air-dried, and threshed. Total grain weight per head, 100-grain weight, and seed color were determined. The seed color was a relative value, ranging from one to white, similar to Combine Kafir 60, to five of dark red, similar to Redlan.

The method used to distinguish between high lysine sorghum grain and normal grain was a modified ninhydrin procedure reported by Mertz et al. (1974). The procedure was modified and these steps were followed: 15 kernels of the grain sorghum were split lengthwise with a single-edged razor blade and placed in 2 x 15-cm Pyrex test tubes. The kernels were

covered with 10 ml of double distilled dionized water and approximately 300 mg of dry ninhydrin-buffer mixture was added. The dry ninhydrin-buffer mixture consisted of 16% ninhydrin, 58% sodium citrate, and 26% citric acid, all in dry form. The test tubes were then placed in a rapidly boiling water bath. The bath held 72 test tubes. The tubes were removed from the bath after seven minutes and then cooled in running tap water for five minutes. The color rankings were determined visually, five being the darkest color formed corresponding to DWM, a known high lysine variety, and one, which corresponded to Redlan, a normal lysine grain sorghum. The method was not quantitative for lysine content, but gave a quick test to distinguish high lysine lines from normal lines. All 2740 F_3 seeds were analyzed with this ninhydrin procedure. Of the 2740 heads analyzed, 925 were from (C X DWM), 826 were from (P X DWM), and 556 were from (R X DWM).

Correlations were run within parental lines (C X DWM, P X DWM, and R X DWM) to compare the variables of seed color, plant height, heading date, 100-grain weight, total head weight, and lysine value.

In selecting lines for field planting in 1976, an index was calculated to obtain 135 acceptable F_2 plants from each of the three parental lines. Only plants that gave lysine values of five were used and the largest 135 values from the following index model were used:

Index = standard head weight + standard 100-grain weight - standard heading date - standard plant height.

Standardized values were obtained by taking the actual values minus the mean of the value, and this difference divided by the standard deviation of the value.

The 1976 field study was planted to F_3 seeds of the 405 F_2 acceptable

plants; F_2 seeds of the 93 F_1 parental plants; the adapted parents (Combine Kafir 60, Redlan, and Plainsman) and Dwarf White Milo; and P-721, a mutant high lysine line from Purdue University. The experiment was laid out in a randomized complete block design with three replications. Each replication consisted of 84 75-cm rows. Each row contained six tiers that were 5-m long with 1-m seperating the tiers. The other two replications were set up in a similar manner.

The seed was treated with Captan and planted with Planet Jr. on May 19-20, 1976. Sufficient seed was planted in each line to obtain normal field stand.

The first six plants to head in each of the 504 lines in Replication I were bagged to insure selfing and height and heading date of the plants were noted. The bagged heads were hand harvested, air-dried, and threshed. Head weight, 100-grain weight, and seed color were also determined similar to the previous year's method.

The lines in replications II and III were hand harvested, threshed, weighed, and measured for moisture content.

Lysine content was again estimated by the method developed by Mertz et al. (1974). 2389 samples were analyzed by the modified ninhydrin procedure; 670 were from (R X DWM), 630 were from (P X DWM), 638 were from (C X DWM), and 451 were parental lines, adapted varieties, DWM, and P-721.

An index was again calculated to rank the plants from each of the three parental crosses. Only plants that gave lysine values of five were included in the ranking.

Index = standard head weight + standard 100-grain weight -
standard heading date - standard plant height.

These standardized values were obtained by taking the actual value minus the mean value of all lines and dividing this difference by the standard deviation of the value. Mean yields of the lines were also used in the determination of the top plants in each parental cross.

Crude protein determination for selected plants was determined by the macro-kjeldahl method and multiplying per cent nitrogen by 6.25.

Selected samples were analyzed for amino acid content with the Beckman Model 120-B Amino Acid Analyzer.

RESULTS

1975 Study

The modified ninhydrin method to distinguish between high lysine sorghum grain and normal grain as reported by Mertz et al. (1974) is a very rapid procedure allowing many samples to be analyzed daily. Up to 288 samples could be analyzed with the limiting factor being the sectioning of the fifteen grains for each sample. A color reaction was observed for each test tube and visually ranked against DWM and Redlan which were run as standards. Figure 1 illustrates the difference in the intensity of color between DWM, a known high lysine sorghum, and Redlan, a normal lysine sorghum. The color reaction from DWM was a very dark purple. This color was given a value of five. The color reaction from Redlan was a very light purple to almost clear. This color was given a value of one. The samples tested were given a lysine value of one to five based on the color reaction. If the samples were darker or lighter then the standards they received the standards value.

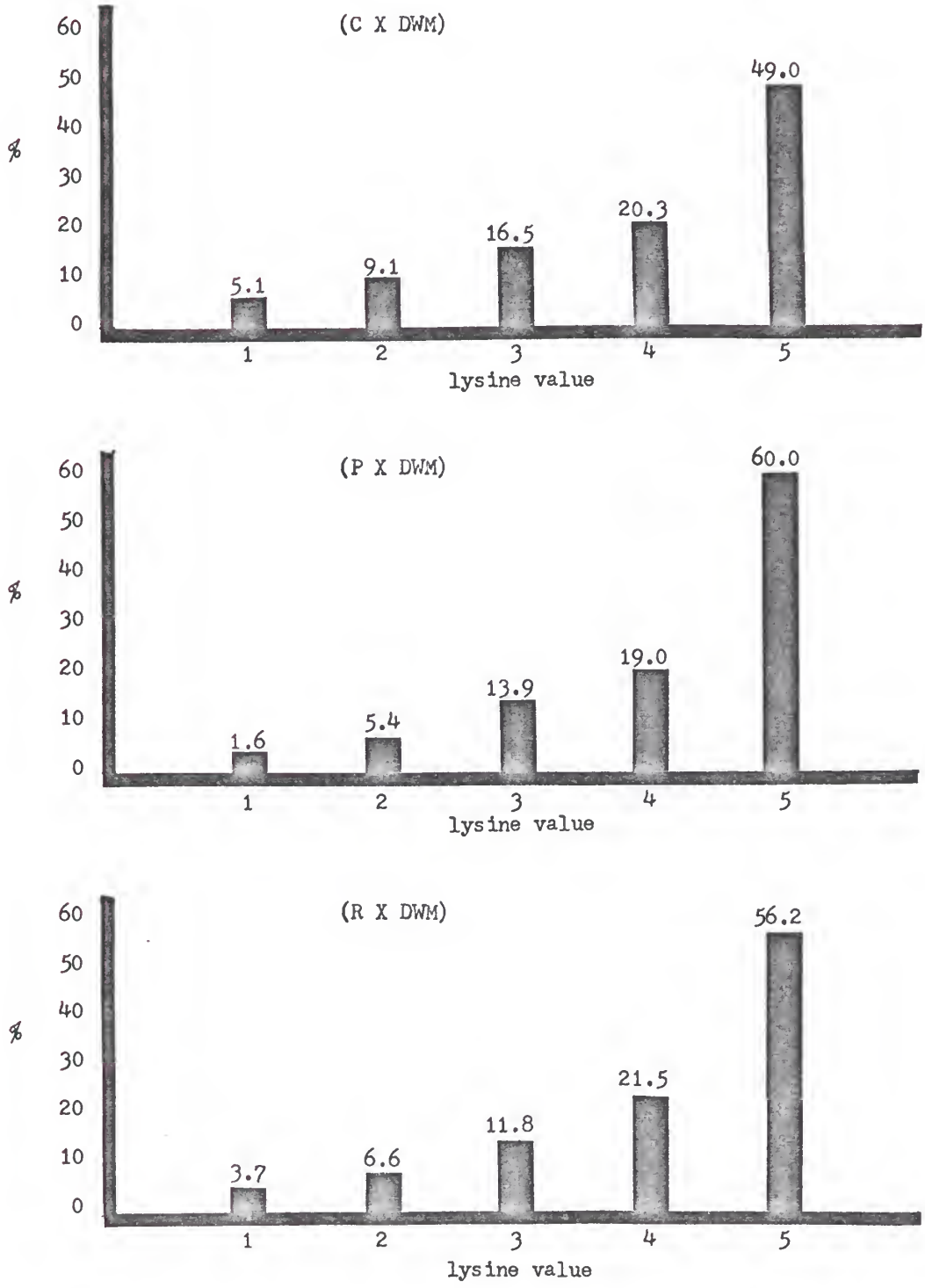
Figure 2 shows the frequency distribution of the lysine value within each cross (C X DWM, P X DWM, and R X DWM). Note that approximately 50% of samples of all three crosses had a lysine value of five.

The other agronomic characteristics that were obtained from the field trial in 1975 were plant height, heading date, 100 grain weight, total head weight, and seed color. Correlation coefficients between these characteristics and lysine value were run using a packaged program available from the statistical laboratory. Plants from each cross were run seperately with the results on Tables 1, 2, and 3.

Table 1 contains the results from cross (C X DWM). Lysine value was significantly correlated with seed color, plant height, heading date

Figure 1. Ninhydrin Color Reaction of Dwarf White Milo (DWM) and Redlan (RED) Grain.

Figure 2. Frequency distribution of lysine value within each cross for 1975 study.



and 100-grain weight. The correlation of lysine value and heading date had a value of .507 which was the highest of all comparisons. Table 2 contains the results from (P X DWM). Lysine value was significantly correlated with plant height, heading date, 100-grain weight, and head weight. Table 3 contains the results from cross (R X DWM). Lysine value was significantly correlated to seed color, plant height, heading date, and 100-grain weight.

The index to provide suitable plants of high lysine and good agronomic traits was developed for the three parental crosses. Mean values of plant height, heading date, 100 grain weight, and head weight from all samples within a parental cross were calculated. The results are shown on Tables 4, 5, and 6. Also, mean values for the same characteristics were calculated from plants that had a lysine value of five. These results are also on Tables 4, 5, and 6. In developing the index the means from all the samples within a parental cross were used instead of the means when the lysine value was five.

In comparing variable means between parental crosses using all samples, one can see that (R X DWM) had the darkest seed color. (P X DWM) had a mean plant height of 126 cm which was the shortest of the other two parental crosses. The earliest mean heading date was the cross (C X DWM). (R X DWM) had the heaviest 100-grain weight, 2.683 g. Also, (R X DWM) had heaviest mean head weight of 50.98 g. Highest mean lysine value was the cross (P X DWM). All three crosses showed similar results when comparing means for all samples to means when lysine value was five. The mean plant height within parental cross increased when samples with lysine of five were used exclusively. Also, heading date, 100-grain weight, and total head weight increased when

Table 1. Correlation coefficients to compare agronomic traits within cross (C X DWM) for 1975 study.

	Seed color	Plant height	Heading date	100 grain weight	Head wt	Lysine value
Seed Color	1.000					
Plant height	.143	1.000				
Heading date	.419	.426	1.000			
100 grain weight	-.317	.106	-.179	1.000		
Head weight	-.179	.291	.094	.520	1.000	
Lysine value	.334	.205	.507	.225	.082	1.000

N = 925

Correlations are significant at α = .01 if value is greater than .083

Table 2. Correlation coefficients to compare agronomic traits within cross (P X DWM) for 1975 study.

	Seed color	Plant height	Heading date	100 grain weight	Head wt	Lysine value
Seed color	1.000					
Plant height	-.027	1.000				
Heading date	.070	.275	1.000			
100 grain weight	-.016	.347	-.046	1.000		
Head weight	-.095	.462	.065	.483	1.000	
Lysine value	-.073	.172	.383	.406	.160	1.000

N = 826

Correlations are significant at α = .01 if value is greater than .096

Table 3. Correlation coefficients to compare agronomic traits within cross (R X DWM) for 1975 study.

	Seed color	Plant height	Heading date	100 grain weight	Head wt	Lysine value
Seed color	1.000					
Plant height	.010	1.000				
Heading date	-.075	.178	1.000			
100 grain weight	-.091	.197	-.204	1.000		
Head weight	-.101	.369	-.065	.389	1.000	
Lysine value	-.111	.184	.329	.348	.072	1.000

N = 939

Correlations are significant at $\alpha = .01$ if value is greater than .076

Table 4. Means from cross (C X DWM) to develop index for 1975 study.

All Samples

Variable	N	Mean	Standard Dev.	Min Value	Max Value
Seed color	925	2.002	1.004	1.000	5.000
Plant height (cm)	925	130.059	29.306	56.000	221.000
Heading date (Julian date)	925	229.643	13.209	203.000	254.000
100 grain wt. (g)	925	2.474	0.654	0.55	3.92
Head wt. (g)	925	42.410	25.038	0.90	144.4
Lysine	925	3.990	1.215	1.00	5.00

Means when Lysine = 5

Variable	N	Mean	Standard Dev.	Min Value	Max Value
Seed color	453	2.397	1.044	1.00	5.00
Plant height (cm)	453	139.331	28.634	60.0	221.0
Heading date (Julian date)	453	236.510	10.878	205.0	254.0
100 grain wt. (g)	453	2.516	.749	.550	3.890
Head wt. (g)	453	41.639	252.778	.90	125.5

Table 5. Means from cross (P X DWM) to develop index for 1975 study.

All Samples

Variable	N	Mean	Standard Dev.	Min Value	Max Value
Seed color	826	3.415	1.257	1.0	5.0
Plant height (cm)	826	126.137	25.440	63.0	243.0
Heading date (Julian date)	826	230.121	9.451	206.0	254.0
100 grain wt. (g)	826	2.566	0.638	0.57	4.4
Head wt. (g)	826	42.026	20.768	1.0	117.1
Lysine	826	4.305	1.004	1.0	5.0

Means when Lysine = 5

Variable	N	Mean	Standard Dev.	Min Value	Max Value
Seed color	496	3.403	1.232	1.0	5
Plant height (cm)	496	129.369	24.895	63.0	193.0
Heading date (Julian date)	496	232.800	8.834	209.0	254.0
100 grain wt. (g)	496	2.746	.636	.57	4.40
Head wt. (g)	496	43.399	21.319	1.0	114.3

Table 6. Means from cross (R X DWM) to develop index for 1975 study.

All Samples

Variable	N	Mean	Standard Dev.	Min Value	Max Value
Seed color	989	3.465	1.316	1.0	5.00
Plant height (cm)	989	136.760	26.777	70.0	214.0
Heading date (Julian date)	989	231.705	9.543	203.0	254.0
100 grain wt. (g)	989	2.683	0.667	0.58	4.56
Head wt. (g)	989	50.983	25.352	.70	151.6
Lysine	989	4.198	1.116	1.00	5.0

Means when Lysine = 5

Variable	N	Mean	Standard Dev.	Min Value	Max Value
Seed color	556	3.388	1.264	1.0	5
Plant height (cm)	556	140.647	26.909	70.0	214
Heading date (Julian date)	556	234.263	9.863	203.0	254.0
100 grain wt. (g)	556	2.838	.718	.58	4.56
Heat wt, (g)	556	50.867	27.141	.70	151.6

samples with lysine of five were used to calculate means.

An index value was calculated for the 2740 F_2 plants analyzed in 1975. The plants with the top 135 index values from each parental cross were selected to be planted in the 1976 field study.

1976 Study

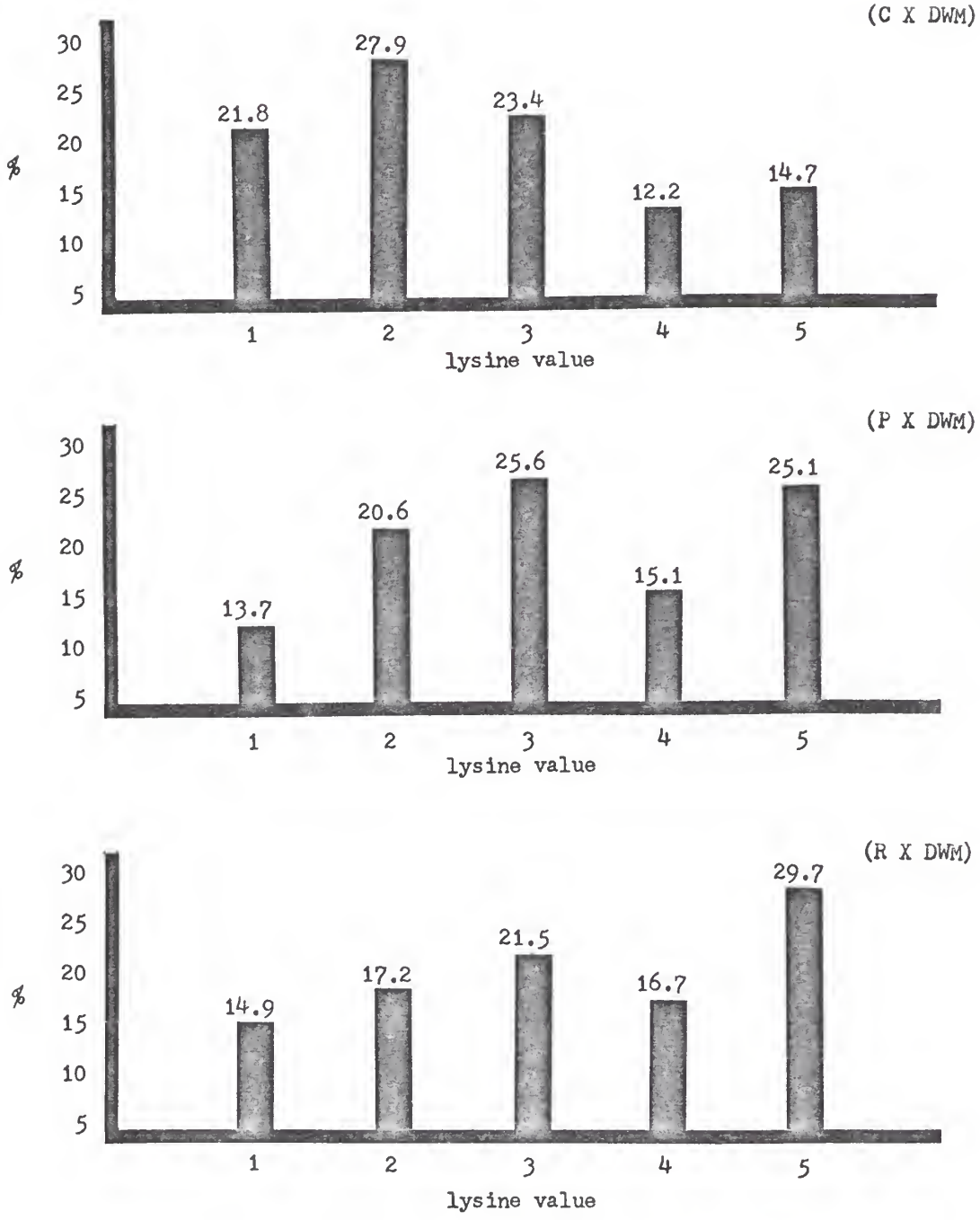
The 1976 study was conducted in similar manner to the 1975 study. The modified ninhydrin procedure to distinguish high lysine was again used to analyze a total of 2389 samples. Figure 3 illustrates the frequency distribution of the lysine value for the 1976 study within crosses. A lysine value of five was found in 14.7% of the (C X DWM) lines, 25.1% of the (P X DWM) lines, and 29.7% of the (R X DWM) lines. These results differ markedly from the frequency distribution of lysine values recorded in 1975. The 1975 study revealed that approximately 50% of the samples within crosses had a lysine value of five.

Correlations among agronomic traits and lysine value were again conducted within parental crosses. Most correlations were significant at alpha = .01, except correlations involving seed color, which were usually nonsignificant. The results are in Tables 7, 8, and 9.

The results of the correlation studies of 1976 were similar to the correlation results of 1975. Within all parental crosses there was a large positive correlation between lysine value and heading date and lysine value and 100-grain weight. These results indicate that a higher lysine value was obtained on plants that headed late and that had large 100-grain weight.

The index using standardized agronomic characteristics was again used to identify the samples with high lysine and good agronomic traits.

Figure 3. Frequency distribution of lysine value within each cross for 1876 study.



Mean values and their standard deviations for samples within each cross for plant height, total head weight, 100-grain weight, and heading date were calculated using packaged programs available from the statistical library. Table 10 contains means for the cross (C X DWM) when all samples were used and also means when only samples that had a lysine value of five were analyzed. Table 11 contains similar data for the cross (P X DWM) and Table 12 contains data for the cross (R X DWM). In calculating the index, only means and standard deviations developed from all samples within each cross were used. The means calculated with samples that had lysine value of five are listed for comparison purposes. As in 1975 study, means for plant height were shorter when only samples with lysine of five were used than when all samples were used. Also means of head weight and 100 grain weight were heavier when lysine was five than when all samples were used.

From the 2389 samples that were analyzed in 1976 only the samples that had a lysine value of five were given an index value. Ninety-four (C X DWM) samples, 158 (P X DWM) samples, and 199 (R X DWM) samples had lysine values of five. From these samples the index was calculated for each cross and ranked.

In selecting for a suitable line that contained high lysine and other good agronomic traits we planned on choosing four lines from each cross that had the highest index value. This was not the sole criterion for selection. In the 1976 study two more replications of each line were harvested and line yields were obtained. The two replication yields were averaged together to obtain an average yield for each line. These average line yields were used as another agronomic trait for lines in replication 1. The following criteria were then followed to obtain

Table 7. Correlation coefficients to compare agronomic traits within cross (C X DWM) for 1976 study.

	Seed color	Plant height	Heading date	100 grain weight	Head wt.	Lysine value
Seed color	1.000					
Plant height	-.145	1.000				
Heading date	.121	-.117	1.000			
100-grain weight	.014	.244	.177	1.000		
Head weight	-.012	.227	.165	.407	1.000	
Lysine value	.142	-.199	.386	.520	.110	1.000

N = 638

Correlations are significant at α = .01 if value is greater than .108

Table 8. Correlation coefficients to compare agronomic traits within cross (P X DWM) for 1976 study.

	Seed color	Plant height	Heading date	100 grain weight	Heat wt.	Lysine value
Seed color	1.000					
Plant height	.056	1.000				
Heading date	-.018	-.309	1.000			
100 grain weight	.097	.163	.286	1.000		
Head weight	-.036	.280	.018	.458	1.000	
Lysine value	.079	-.264	.566	.590	.168	1.000

N = 630

Correlations are significant at α = .01 if value is greater than .106

Table 9. Correlation coefficients to compare agronomic traits within cross (R X DWM) for 1976 study.

	Seed color	Plant height	Heading date	100 grain weight	Head wt.	Lysine value
Seed color	1.000					
Plant height	-.097	1.000				
Heading date	-.009	-.320	1.000			
100 grain weight	.066	.050	.184	1.000		
Head weight	.046	.304	-.021	.346	1.000	
Lysine value	-.012	-.324	.467	.610	.096	1.000

N = 670

Correlations are significant at α = .01 if value is greater than .097

Table 10. Means from cross (C X DWM) to develop index for 1976 study.

All Samples

Variable	N	Mean	St Dev	Min Value	Max Value	Std Error of Mean
Height (cm)	638	128.263	30.012	60.0	200.0	1.188
Head wt (g)	638	38.540	22.793	1.84	123.19	0.902
100 seed wt (g)	638	2.927	0.684	0.85	4.56	.027
Color	638	1.113	0.326	1.0	3.00	.013
Lysine value	638	2.702	1.333	1.0	5.0	.053
Heading date (Julian date)	638	217.577	11.572	209.0	241.0	.458

Means when lysine value = 5

Variable	N	Mean	St Dev	Min Value	Max Value	Std Error of Mean
Height (cm)	94	115.447	23.516	62.0	172.0	2.426
Head wt (g)	94	39.576	26.067	4.92	119.24	2.689
100 seed wt (g)	94	3.395	0.549	1.496	4.42	0.057
Color	94	1.223	.444	1.0	3.0	0.045
Heading date (Julian date)	94	225	7.951	208.0	241.0	0.820

Table 11. Means from cross (P X DWM) to develop index for 1976 study.

All Samples

Variable	N	Mean	St Dev	Min Value	Max Value	Std Error of Mean
Height (cm)	630	124.257	28.790	62.0	190.0	1.147
Head wt (g)	630	48.062	20.785	3.49	115.01	0.828
100 seed wt (g)	630	3.046	0.737	0.95	4.658	0.029
Color	630	2.222	1.091	1.00	5.00	0.043
Lysine	630	3.173	1.371	1.0	5.00	0.055
Heading date (Julian date)	630	220.556	7.582	201.0	241.0	0.302

Means when lysine = 5

Variable	N	Mean	St Dev	Min Value	Max Value	Std Error of Mean
Height (cm)	158	108.437	23.938	62.0	178.0	1.904
Head wt (g)	158	49.543	19.812	4.76	115.01	1.576
100 seed wt (g)	158	3.588	0.626	1.868	4.625	0.050
Color	158	2.323	1.113	1.00	5.00	0.089
Heading date (Julian date)	158	227.563	7.329	208.0	241.0	0.583

Table 12. Means from cross (R X DWM) to develop index for 1976 study.

All Samples

Variable	N	Mean	St Dev	Min Value	Max Value	Std Error of Mean
Height (cm)	670	129.676	30.404	60.0	200.	1.175
Head wt (g)	670	54.605	24.882	0.26	143.15	0.961
100 seed wt (g)	670	3.236	0.729	0.0	5.06	0.028
Color	670	2.539	1.324	1.0	5.0	0.051
Lysine	670	3.291	1.429	1.0	5.0	0.055
Heading (Julian date)	670	222.399	6.914	206.0	241.0	0.267

Means when lysine = 5

Variable	N	Mean	St Dev	Min Value	Max Value	Std Error of Mean
Height (cm)	199	116.513	23.768	60.0	172.0	1.685
Head wt (g)	199	53.433	24.355	1.69	113.03	1.726
100 seed wt (g)	199	3.723	0.549	0.0	4.78	0.039
Color	199	2.533	1.329	1.00	5.00	0.094
Heading (Julian date)	199	226.015	6.350	212.0	241.0	0.450

four lines from each cross. Mean line yields were calculated within crosses and, for a line to be selected, it had to have a line yield better than the average for all lines in that cross. The top four lines selected in each cross had the highest index values among all other plants and also had line yields better than the average. Table 13 contains the selected four lines from each parental cross chosed by the above method. The table contains the index value and the agronomic traits that were used to develop the index. Table 13 also contains characteristics of the four parents and P-721, a known high lysine sorghum.

Table 14 illustrates the line yields of the selected lines and also the average line yields within the parental cross. The line yields of the four parents and P-721 are also presented.

The 17 selected lines (12 crosses, four parents, and P-721) were analyzed further by obtaining protein content by macro-Kjeldahl method. The results from this test are on Table 15. The line with the highest protein concentration was (R X DWM 6-11-21-6) with a protein of 14.78%; however, only Combine Kafir 60 had a very low protein concentration (10.77).

The 17 selected samples were analyzed for amino acid content with the Beckman Model 120-B Amino Acid Analyzer. The results are in Table 16. Only P-721, a known high lysine grain sorghum from Purdue, gave a high lysine value (2.63g per 100g protein). All the others had lysine values which could be considered in the normal range for grain sorghum. Dwarf White Milo, the grain sorghum from the Republic of Sudan, had a lysine value of 1.55g per 100g protein, which is considerably different than the 3.01g per 100g protein that was reported by Hoseney et al. (1974).

Table 13. Index value and agronomic traits for selected samples for 1976 study.

Variety	Line-Plant	Heading date	Lysine value	Height	Head weight	Seed weight	Color	Index
		Julian day		cm	g	g		
CD	2-11-020-3	222	5	119	102.80	3.893	1	4.160
CD	1-16-020-1	232	5	90	119.24	3.170	1	3.925
CD	1-03-004-1	226	5	101	79.93	3.790	2	3.260
CD	2-20-013-2	220	5	116	61.67	4.014	1	2.805
PD	1-13-001-4	213	5	80	53.87	4.220	1	4.406
PD	2-16-014-6	214	5	134	73.56	4.322	2	3.484
PD	2-11-004-5	220	5	93	79.54	3.578	1	3.395
PD	2-02-033-2	224	5	98	87.65	3.713	4	3.267
RD	1-09-002-1	220	5	98	112.69	3.910	2	4.648
RD	1-02-005-4	220	5	130	98.22	3.940	1	3.055
RD	1-11-021-6	215	5	138	75.63	4.250	1	3.032
RD	1-09-012-6	218	5	172	104.34	4.490	2	2.963
CK 60	plant 4	208	1	98	33.03	1.77	1	
Redlan	plant 6	220	1	100	82.97	3.10	4	
Plainsman	plant 6	214	1	90	39.26	2.14	1	
DWM	plant 5	228	5	120	41.75	4.20	1	
P-721	plant 1	213	5	123	24.13	2.31	1	

Table 14. Mean grain yield of selected sorghum lines for 1976 study.

Variety	Plant	Line	Line-Yield	Ave. Line-Yield
			kg/ha	kg/ha
CD	2-11-020-3	CD 2-11-020	4776	3506-5
CD	1-16-020-1	CD 1-16-020	4316	
CD	1-03-004-1	CD 1-03-004	4039	
CD	2-20-013-2	CD 2-20-013	5961	
PD	1-13-001-4	PD 1-13-001	3770	3691.4
PD	2-16-014-6	PD 2-16-014	4097	
PD	2-11-004-5	PD 2-11-004	3780	
PD	2-02-033-2	PD 2-02-033	3851	
RD	1-09-002-1	RD 1-09-002	6798	4181.0
RD	1-02-005-4	RD 1-02-005	5694	
RD	1-11-021-6	RD 1-11-021	4948	
RD	1-09-012-6	RD 1-09-012	5672	
CK60			3248	
Redlan			3240	
Plainsman			3381	
DWM			2408	
P721			2035	

Table 15. Head weight, 100-grain weight, and protein concentration of selected lines for 1976 study.

Line	plt#		Head wt	100 grain weight	Protein
			g	g	%
1	RxD 1-9-2	1	112.69	3.91	14.68
2	RxD 1-9-12	6	104.34	4.49	13.52
3	RxD 6-11-21	6	75.63	4.25	14.78
4	CxD 1-3-4	1	79.93	3.79	14.27
5	CxD 1-16-20	1	119.24	3.17	12.44
6	CxD 2-24-13	2	61.67	4.014	13.70
7	PxD 2-16-14	6	73.6		
8	PxD 2-11-4	5	79.54	3.578	14.02
9	Redlan	6	82.97	3.10	13.19
10	DWM	5	41.75	4.20	13.86
11	P 721	1	24.13	2.31	14.27
12	CxD 2-11-20	3	102.80	3.93	12.18
13	RxD 1-2-5	4	98.22	3.94	14.52
14	PxD 1-13-1	4	53.87	4.22	13.71
15	CK60	4	33.03	1.77	10.77
16	PxD 2-2-33	2	37.65	3.713	13.19
17	Plainsman	6	39.26	2.14	13.10

Protein is N x 6.25 on oven dry samples

Table 16. Lysine content by amino acid analysis of selected samples for 1976 study.

Line	Plant No.	Lysine
		g/100g protein*
R X DWM 1-9-2	1	1.48
R X DWM 1-9-12	6	1.79
R X DWM 6-11-21	6	1.39
C X DWM 1-3-4	1	1.74
C X DWM 1-16-20	1	1.65
C X DWM 2-24-13	2	1.67
P X DWM 2-16-14	6	1.46
P X DWM 2-11-4	5	1.54
Redlan	6	1.45
DWM	5	1.55
P-721	1	2.63
C X DWM 2-11-20	3	1.80
R X DWM 1-2-5	4	1.42
P X DWM 1-13-1	4	1.47
CK60	4	1.79
P X DWM 2-2-33	2	1.55
Plainsman	6	1.58

*Corrected to 100% recovery on Kjeldahl Protein Results.

DISCUSSION

The purpose of this study was to transfer the high lysine trait from Dwarf White Milo to adapted grain sorghum varieties. Dwarf White Milo was reported by Hoseney et al. (1974) to contain 3.01g of lysine per 100 g of protein. Also, Dwarf White Milo had normal appearing grain, normal protein concentration, and hard endosperm. Assuming Dwarf White Milo to be a high lysine grain sorghum, crosses were made with three adapted normal lysine grain sorghums; Combine Kafir 60, Plainsman, and Redlan.

In selecting a technique to screen for high lysine grain sorghum, we looked for one that was inexpensive to run, rapid because of the quantity of samples to be tested, and reliable in distinguishing between high and normal lysine grain sorghum. Numerous techniques have been used that quantitatively measure lysine concentration by colorimetry, but these are too slow and too expensive for our effort.

Mertz et al. (1973) reported a technique to use ninhydrin as a reagent to distinguish between high lysine and normal lysine grain. Ninhydrin binds with the free amino groups (both alpha and epsilon) on protein and also with the amino groups from free amino acids. This procedure was rapid and inexpensive, which was a necessity for the number of samples that needed to be analyzed. The resulting color reaction showed a great range of color; from very dark blue in Dwarf White Milo to almost clear in the normal lysine variety, Redlan (Figure 1). This indicated that Dwarf White Milo had more free amino acid and more epsilon amino groups in the protein, thus a higher than normal lysine concentration. The results of the amino acid analysis found on Table 16

shows Dwarf White Milo only to have 1.55g per 100g protein, which is not any higher than that found in normal lysine grain sorghums. This is in disagreement with the results of Honeney et al. (1974), which stated that Dwarf White Milo had a lysine concentration of 3.01g per 100g protein.

There are three possible conclusions one could make of this disagreement. (1) The results of the latest amino acid analysis of Dwarf White Milo were incorrect. (2) The results of Hoseney et al. (1974) were incorrect. or (3) Dwarf White Milo did indeed have a high lysine concentration when Hoseney et al. (1974) analyzed it, but three generations of selfing and different climatic conditions caused Dwarf White Milo to fail to express its high lysine trait. The third conclusion possibly is the most realistic because the Dwarf White Milo seed Hoseney et al. (1974) analyzed was obtained from the Republic of Sudan and had never been grown in this country. The environment to which the seed was exposed was different than the environment of the United States. The seed in which the latest amino acid analysis was tested had been grown in Manhattan for three generations, one in the greenhouse and two in field studies. Both the 1975 and 1976 studies were grown under adverse conditions (very wet at planting time and then drying out and being very droughty through the rest of the growing season). These adverse conditions could have prevented the high lysine trait from being expressed. Our Dwarf White Milo samples also contained higher protein concentration than that from the Republic of the Sudan, probably because of differences in nitrogen fertility, between the two locations. The higher protein in our samples might have caused the lower lysine concentration. Also, the other lines selected from the initial crosses

in 1974 showed no increase in lysine concentration. Either the high lysine trait was lost when the cross was made or the ninhydrin method was incorrect. The results of the ninhydrin method when compared with the amino acid analysis show a distinction between high and normal grain sorghum varieties. The 12 samples selected from crosses that were analyzed for amino acid concentration were chosen because the ninhydrin method showed high lysine in each of the samples. Table 16 shows the results of the amino acid analysis for these 12 ninhydrin method high lysine varieties. The lysine content of all twelve are well within the range for normal lysine grain sorghum varieties.

Further study should be conducted on the Dwarf White Milo to possibly find if the high lysine trait that was present when Hoseney et al. (1974) analyzed it could be recovered and then a genetic pattern for inheritance could be found.

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TRANSFER OF HIGH LYSINE TRAIT TO ADAPTED SORGHUM VARIETIES

by

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A grain sorghum (Sorghum bicolor L. Moench.) introduction (PI 400226) from the Republic of the Sudan was observed to contain few endosperm protein bodies. That trait is compatible with high grain lysine; amino acid analysis measured a lysine content of 3g per 100g protein. Other seed traits were normal and the endosperm was hard. The high lysine introduction was crossed with the adapted sorghum varieties 'Combine Kafir 60', 'Plainsman', and 'Redlan'. In 1975, approximately 3000 F_3 lines containing a range of agronomic traits, seed color and size, and maturity were evaluated for lysine content by the ninhydrin method. The lines were evaluated further by formulating an index value for each line which was a measure of their agronomic worth. 135 lines of each parental cross with the highest index value were grown in a field study in 1976. Approximately 2400 F_4 lines were evaluated for lysine content by the ninhydrin method and other agronomic traits. Twelve lines were selected for high lysine and suitable agronomic traits by an index similar to the one used in 1975. These twelve lines and varieties; (PI 400226), Combine Kafir 60, Plainsman, Redlan, and P-721 (a high lysine line from Purdue) were analyzed for lysine content by amino acid analysis. Results showed that only P-721 had a higher than normal lysine content. All other lines, including introduction (PI 400226), had lysine contents within normal levels for grain sorghum. Environmental interaction with the lysine trait could have caused introduction (PI 400226) to fail to express the trait. Amino acid analysis of the twelve selected high lysine lines and of introduction (PI 400226) show that these lines contained normal concentrations of lysine while the ninhydrin method indicated that these thirteen lines had a higher than normal lysine concentration.