CHEMICALLY INDUCED HIGH LYSINE MUTANTS IN *SORGHUM BICOLOR* (L.) MOENCH

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ABSTRACT

Mohan, Daya Prakash. Ph.D., Purdue University, May 1975. Chemically induced high lysine mutants in Sorghum bicolor (L.) Moench. Major Professor: J. D. Axtell.

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

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

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

The inheritance data suggest that the opaque character is simply inherited as a partial dominant. A preliminary test for allelism indicates that this gene is nonallelic with the (hl) gene in IS11758.

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

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

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

Isonitrogenous rations (10% protein) of the best Ethiopian high lysine line from the world collection (IS11758), and the P721 opaque were compared in feeding trials with weanling rats and chicks. IS11758 was found to be superior. Gain in weight of rats and chicks on the P721 opaque ration was 65% and 75% of the gain on IS11758 ration, respectively.

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

The P721 opaque possess many agronomic characteristics which should make it useful in the improvement of sorghum lines and hybrids. It is photoperiod insensitive, short enough to allow combine harvesting, and has

wide agronomic adaptability. The seed of P721 is plump with a white pericarp, but of greatest importance is the high lysine content coupled with the low isoleucine-leucine ratio. The P721 opaque should prove valuable in both temperate and tropical regions of the world.

INTRODUCTION

More important than the discovery and direct use of opaque-2 (Mertz, Bates, and Nelson; 1964), and floury-2 genes (Nelson, Mertz, and Bates; 1965), was perhaps a realization of the possibility of genetic variation of endosperm protein composition. As a consequence, investigations were carried out on other cereals on the supposition that similar variations could be expected to occur naturally or could be induced.

One high lysine mutant has been found in the world collection of barley (Munck, Karlsson, and Hagberg; 1969), and another in sorghum (Singh and Axtell, 1973). Induced high lysine mutants have been reported in barley by Doll (1972) and Ingversen et al. (1973).

This study deals with induced high lysine mutants in grain sorghum. The discovery of one of these mutants designated P721 is likely to be useful in improving the nutritional quality of grain sorghum in temperate as well as tropical regions of the world.

Sorghum is the fourth most important cereal crop of the world. The protein quality of sorghum grain is poor when compared to that of other major cereals. The first limiting amino acid in sorghum protein is lysine. Therefore, the availability of genes which improve the lysine content of sorghum endosperm protein is the first requisite of any breeding program for quality improvement of grain sorghum.

The objectives of this study were:

1) to identify high lysine opaque mutants with improved protein quality among progeny of sorghum seeds treated with the chemical mutagen diethyl sulfate.

2) to evaluate the biological value of grain produced from such mutants with weanling rat and chick feeding trials.

3) to determine the mechanism of inheritance of the selected high lysine character.

4) to compare the internal morphology of endosperm cells of opaque and normal sib kernels.

REVIEW OF LITERATURE

Genetic Control of Protein Quality and Content

The first report of a mutant gene or genes in grain sorghum which improved protein quality was made by Singh and Axtell (1973). Seeds from over 9000 lines in the world sorghum collection were classified for floury endosperm phenotype. Two Ethiopian lines IS11167 and IS11758 were found with two-fold increases in lysine content as percent of sample when compared to normal sorghum. The hl gene is inherited as a simple recessive. An increase in lysine content of IS11758 or IS11167 was caused by reduction in the proportion of alcohol soluble proteins or kafirins and an increase in percent germ over the normal.

Mertz et al. (1964) and Nelson et al. (1965) first reported that the mutant genes opaque-2 and floury-2 in maize changed the amino acid composition of endosperm tissue homozygous for these genes. The search at that time was for a mutant that would block the production of the prolamine fraction (the zeins) and possessed opaque phenotype (Nelson, 1966). Cereals with high prolamine content like barley and sorghum could have a possibility of similar mutations as in maize (Nelson, 1966). Hagberg and Karlsson (1969) reported an accession number CI3947 in the world barley collection which showed high levels of lysine content. The inheritance of high lysine character of CI3947 (also named 'Hiproly') was

reported by Munck et al. (1969).

Doll (1972) found two high lysine mutants in barley using ethyl methyl sulfonate. The barley mutant Riso 1508 was an ethylenoimine induced mutant of Bomi variety (Ingversen, 1973).

Virupaksha and Sastry (1968) reported a sorghum 160 Cernum with high lysine content. This represented analysis of a single sample without inheritance data. They also reported fractionation data using the modified Osborne and Mendel (1914) technique for two sorghum varieties, confirming that the alcohol soluble prolamine fraction (kafirin) like the zein fraction of maize, was low in lysine content. Swaminathan et al. (1969, 1970) fractionated samples of six sorghum varieties and Jambunathan and Mertz (1973) fractionated samples of four varieties using the procedure of Landry and Moureaux (1970). Their conclusions were essentially similar.

McWhirter (1971) identified opaque-2 in maize which was similar to $\underline{o}_2$ and $\underline{fl}_2$ in that there is decrease in prolamine fraction and increase in glutelin fraction in the endosperm. The characteristics of these mutants are tabulated in Table 1.

Other endosperm mutants which improve the lysine, tryptophan and decrease leucine content in maize endosperm protein are brittle-2, brittle-1, shrunken-2, shrunken-4 and sugary-1 (Barbosa, 1971; Misra et al. 1972a, 1972b, 1974a, 1974b; Glover et al. 1972). The nutritional quality of maize has also been improved in double-mutant combinations such as sugary-1 opaque-2, shrunken-2 opaque-2, brittle-2 opaque-2, brittle-1 opaque-2 (Barbosa, 1971; Glover, 1971, 1972, 1973). (Glover et al. 1972; Glover and Tosello, 1973) reported that sugary-2 opaque-2 combination has improved density, protein quality, digestibility and biological value compared to opaque-2.

Table 1. Defatted endosperm or whole seeds amino-acid composition of major genes in various cereal crops which drastically change composition of endosperm proteins compared to their normal counterparts expressed as g per 100 g of protein.

Crop		Lys	Tryp	Arg	Asp	Gly	Meth	Glu	Ala	Leu	Phe	Tyr	His	Thr	Ser	Pro	Val	Cyst	Iso	Protein	Inheritance
Endosperm of Maize genotype (Nelson, 1969)	W64+	1.6	0.3	3.4	7.0	3.0	2.0	26.0	10.1	18.8	6.5	5.3	12.9	3.5	5.6	8.6	5.4	1.8	4.5	12.7	—
	W64o_2	3.7	0.7	5.2	10.8	4.7	1.8	19.8	7.2	11.6	4.9	3.9	3.2	3.7	4.8	8.6	5.3	0.9	3.9	11.1	recessive
	fl_2	3.3	0.8	4.5	8.1	3.7	3.2	19.1	8.0	13.3	5.1	4.5	2.2	3.3	4.8	8.3	5.2	1.8	4.0	13.6	semi-dominant
(Misra et al. 1972)	W22+	2.3	0.4	3.7	6.7	4.0	3.2	27.4	8.5	15.9	6.7	5.8	3.6	4.0	5.4	11.5	5.7	2.4	4.3	8.5	—
	W22o_7	3.8	0.7	5.2	9.4	5.2	3.2	25.5	7.9	12.5	5.2	4.9	4.1	4.4	5.6	11.0	6.7	1.9	4.3	7.3	—
Barley genotype whole seeds (Munck, 1972)	(normal)	3.4	-	4.6	6.0	3.6	1.2	26.8	3.8	6.7	5.9	2.8	2.1	3.4	4.3	12.6	4.8	1.1	3.7	15.7	—
	Hiproly	4.2	-	4.9	6.8	3.9	1.5	23.9	4.4	7.1	5.9	2.8	2.2	3.6	4.4	11.3	5.3	0.9	3.9	17.0	recessive
Barley genotype whole seeds (Ingversen et al. 1973)	Variety Bomi (normal)	4.7	-	5.9	7.5	4.8	1.8	30.5	5.1	9.0	6.4	2.9	2.8	4.0	5.3	13.8	6.4	1.5	4.4	-	—
	Riso mutant 1508	7.1	-	8.3	11.6	7.1	2.0	21.3	6.9	8.8	5.3	3.2	3.6	5.4	5.8	7.8	6.9	1.3	4.3	-	recessive*
Sorghum whole seeds (Singh, 1973)	(normal)	2.1	1.3	3.6	7.8	3.1	1.8	23.2	9.9	14.3	5.2	4.5	2.0	3.3	4.5	8.2	5.4	1.5	4.1	12.6	—
	hl	3.1	1.2	4.8	7.4	3.7	2.1	22.4	9.2	13.2	5.4	4.3	2.1	3.2	4.2	6.7	5.2	1.9	4.1	17.2	recessive

* (Doll, 1973).

Nutritional Studies

Sorghum grain is used as human food. The quality of sorghum protein is poor. Pond, Hillier, and Benton (1958), and Howe, Jansen, and Gilfillan (1965), showed in trials with weanling rats that lysine and threonine are first and second limiting amino acids in sorghum. Pellagra found in the Deccan plateau in India has been ascribed to high leucine to isoleucine ratio in grain sorghums eaten as a staple food there (Gopalan, 1968). Morton (1970) has tentatively linked higher incidences of esophageal cancer with consumption of sorghums rich in tannin. Catechin tannins can also cause inflammation of gastro intestinal tract (Morton, 1970).

Howe et al. (1965) surveyed the protein efficiency ratio (PER) values of nine unsupplemented, ground, whole cereal grains fed to rats on diets containing 7.8 to 10% protein and found oats to have the highest value (1.8) when compared to casein (2.5). Barley, (1.6), rice (1.5), maize (1.4), bulgur (1.5), rye (1.3), wheat (.90), sorghum (.6), and millet (.2) were below oats in PER value. The data of Mertz et al. (1965), and Mertz (1972) indicate PER values calculated for young rats fed on opaque-2 maize proteins have a food value equal to casein control.

The reduced level of leucine in opaque-2 corn may also contribute to its superior nutritional value. Gopalan, Belavady, and Krishnamurthi (1969), showed that high levels of leucine found in normal corn and sorghum produce pellagra in humans when these cereals form major part of the diet.

The opaque-2 corn protein is of superior nutritive value when compared to normal corn. This has been demonstrated for humans (Bressani,

1966; Clark et al. 1967; Harpstead and Sarria, 1968), for pigs (Pickett, 1966; Cromwell et al. 1967), for rats (Maffia, 1973). Nutritional evaluation of floury-2 corn proved it superior to normal corn for humans (Harpstead and Sarria, 1969) for chicks (Cromwell et al. 1968) and for rats (Veron, 1967). However, Gipp, Cline and Rogler (1968), reported that floury-2 corn appeared to be intermediate to normal and opaque-2 corn when fed to rats. Nelson and Mertz (1973) report that nutritive value for weanling rats of two high protein high lysine floury-2 maize hybrids was not superior to opaque-2 hybrids of lower protein and lysine content.

Preliminary data with double mutants show brittle-2 opaque-2 and sugary-2 opaque-2 have good biological value (Mertz, 1972).

Opaque-2 protein digestibility in rats was similar to that of Indiana hybrid corn 453 (Mertz et al. 1965).

Munck (1972) cites personal communication of Eggum, page 93, reporting that best high lysine barley lines have net protein utilization (NPU) in rat tests equal to opaque-2 and superior to floury-2.

True digestibility is slightly increased in all high lysine lines compared with normal control (Munck, 1972, page 92).

The two Ethiopian high lysine lines IS11758 and IS11167 discovered by Singh and Axtell (1973) had a PER value of 2.06 and 1.70, respectively, in contrast to normal sorghum which had a PER value of 0.61 and casein control 2.20 when fed to rats.

The hl gene in IS11758 sorghum has superior nutritive value for chicks (Featherston et al. 1975).

The high lysine gene in sorghum is associated with relative larger germ size, higher oil content, lower 100 seed weight in the case of IS11758 and is equal to normal sorghum in the case of IS11167 (Singh and Axtell, 1973).

The low leucine to isoleucine ratio of sorghum in IS11167 and IS11758 when compared to normal sorghums may also contribute to superior nutritional value because of antipellagragenic properties. This has been confirmed for opaque-2 corn (Gopalan et al. 1969).

Oswalt (1973) showed that rat weight gains were positively correlated with lysine content of grain in rations made from genotypes containing low catechin equivalent concentrations. However, this was not true for genotypes high in catechin equivalent concentrations. He therefore concluded that to evalute nutritional quality of grain sorghum, genotypes containing low and high concentration of catechin equivalents need to be investigated separately.

Effect of Agronomic Factors on Grain Protein

Application of nitrogen fertilizer increased kafirin level as shown by Waggle et al. (1967) and Campbell and Pickett (1968). Schneider, Early and DeTurk (1952), showed similar results for corn, that is, the zein fraction in corn increased more than any other fraction as a result of nitrogen fertilization.

Development and Distribution of Proteins in Grain

Taira (1964) showed that as the grain progresses from the milky to the mature stage there is a rapid increase in the amount of prolamine and glutelins. In contrast the albumin and globulin proteins which predominate in early stages of seed development constitute a smaller fraction of the total protein of mature seed.

For a similar review for barley the reader is referred to Munck (1972).

The Distribution of Protein Bodies in Sorghum Endosperm

Protein bodies have been reported from ungerminated seeds, both dicots and monocots. In cereals they have been reported in corn (Christianson et al. 1969), wheat (Graham, Morton, and Raison 1963; Graham et al. 1964; Morton, Palk, and Raison 1964), rice (Mitsuda et al. 1969), barley (Ory and Henningsen, 1969; Tronier and Ory, 1970; Tronier, Ory, and Henningsen 1971), and from sorghum (Wolf and Khoo, 1970; Seckinger and Wolf, 1973).

In cereals the picture of protein bodies are not similar as in oilseeds but thus far has different types of associated substructures (Graham et al. 1963; Khoo and Wolf, 1970; Mitsuda et al. 1969; Ory and Henningsen, 1969; Seckinger and Wolf, 1973).

This review will be confined to sorghum and corn protein bodies.

In sorghum the aleurone layer of the endosperm is rich in albumins and globulins (Wall, 1970). Just inside the aleurone at the periphery of horny endosperm is a dense layer of cells rich in kafirin (Watson et al. 1955). There are larger amounts of protein in the peripheral region of the endosperm (Wall, 1967). Sorghum endosperm protein is similar to that of corn, because of large percentage of prolamine (Hansen, Brimhall, and Sprague 1946; Jones and Beckwith, 1970). Improved lysine types have been found from world sorghum collection (Singh and Axtell, 1973). There was a marked reduction in size of protein bodies in opaque-2 (Wolf, Khoo, and Seckinger; 1967), a major site of zein in maize (Duvick, 1961). Wall and Blessing (1969), reported that

sorghum endosperm protein contains protein bodies which could be deposits of prolamine. Seckinger and Wolf (1973), reported that concentration of protein within endosperm of grain sorghum varies with texture. Vitreous cells of subaleurone layer contain mostly protein with little or no starch. While the opposite is true in floury endosperm cells that there is loose starch with small percentage of protein (Seckinger and Wolf, 1973).

Correlating size of protein bodies with prolamine content can provide an indirect measure of lysine content (Seckinger and Wolf, 1973), that the lysine level is negatively correlated with the presence of protein bodies in case of high lysine sorghum IS11758 and IS11167 where protein bodies are absent or not visible in the light microscope (Clark, 1975) in maize (Choe, Cumbie, and Zuber 1974; Baenziger, 1974).

Rooney and Sullins (1973), reported the structure of high lysine sorghum SC1030 of Ethiopian origin, discovered by Rosenow, with a scanning electron microscope. The high lysine kernels had a significant reduction in the number and size of protein bodies when compared to its normal counterpart.

SECTION I

INITIAL SURVEY OF THE INDUCED MUTANTS FOR OPAQUE ENDOSPERM MUTANTS

Materials and Methods

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

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

Parental Germplasm

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

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

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

Mutagen Treatments

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

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

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

Each head was threshed individually, cleaned and packeted in a coin envelope. A random sample of seeds was taken from each of the coin envelopes and examined over a light box for opaque mutant kernels. Figure 1 shows seeds from one of these heads in bright light and Figure 2 shows the same seeds over the light box. It is obvious that these seeds are segregating for opaque mutant kernels. Approximately 14,000 heads were screened before the May, 1973 planting at Lafayette and another 9000 heads were screened before the 1973-74 Puerto Rico winter planting.

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

Results and Discussion

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

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

Figure 1

Mutant seeds in bright light. (Normal vitreous seeds and P721 opaque seeds).

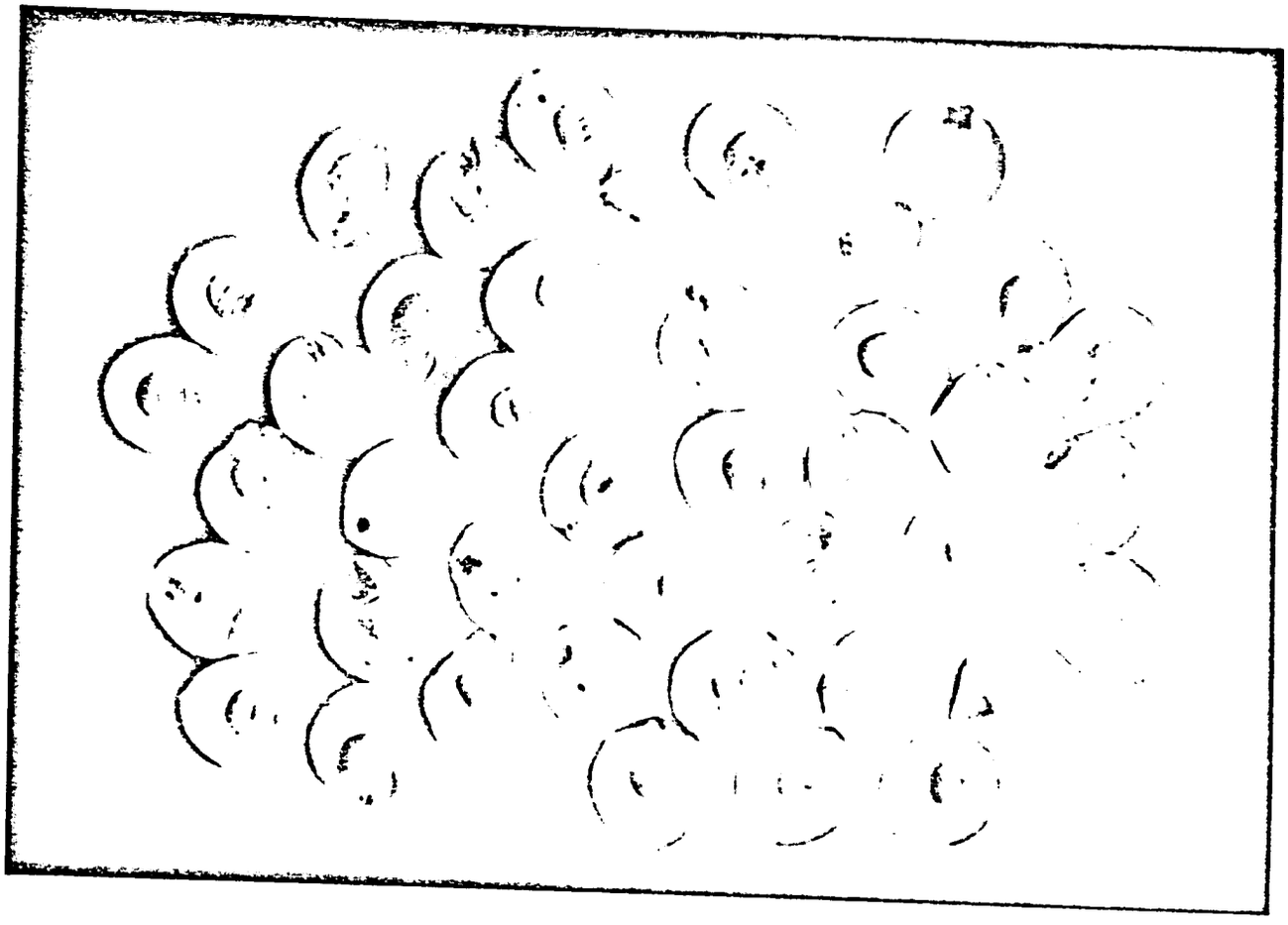

Figure 1

Figure 2

Mutant seeds over a light box showing segregation into vitreous (normal) and opaque classes. (The P721 seeds used are only for the purpose of illustration and not to prove segregation ratios).

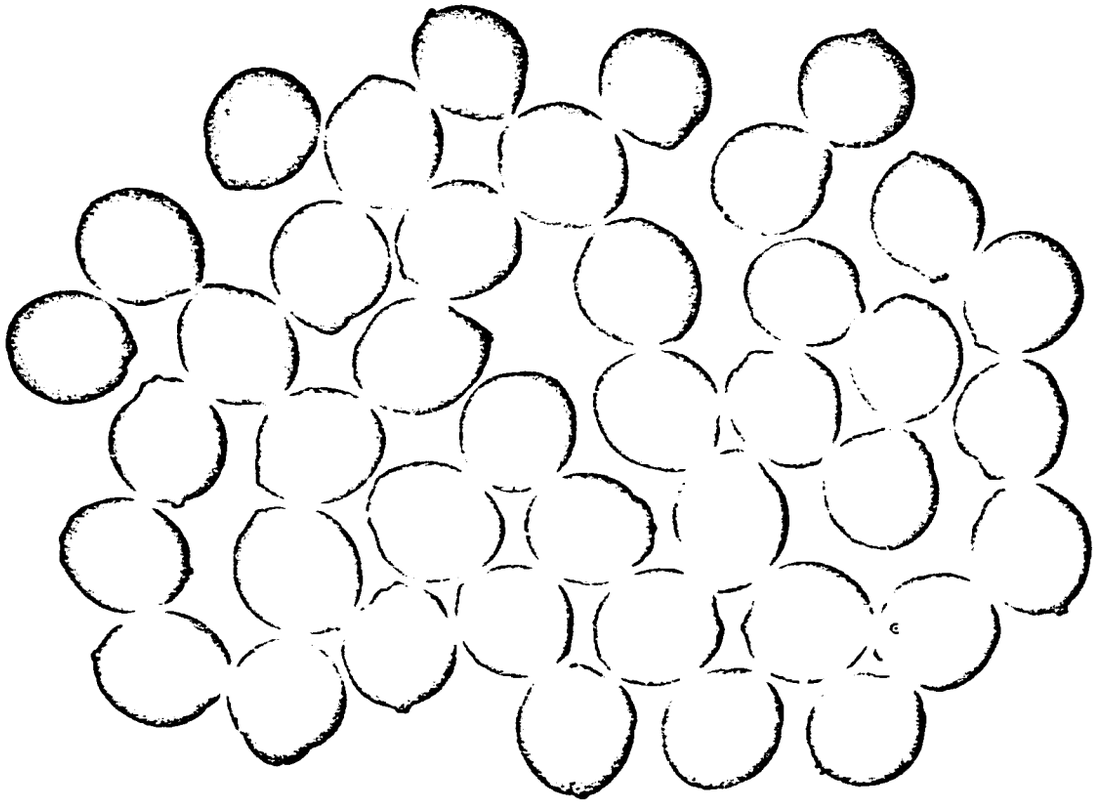

Figure 2

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

Table 2 illustrates the distribution of putative opaque mutants in terms of their relative increase in lysine content when compared to normal vitreous control sib seeds. As a control for the homozygous opaque seeds an average of all (445) normal sibs was taken which was equal to 14.4% protein and 1.9% lysine (as percent of protein). Lysine expressed as percent of sample was equal to .274. The comparison for increase in lysine content for homozygous opaques was with this number.

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

This study is confined to those opaque mutants which have had an increase of lysine concentration of more than 50%. Twenty-eight of these mutants (Table 3) were planted in the greenhouse or field at Lafayette or in Puerto Rico. Seven of these mutants were not planted because the seed size was too small and the high protein levels were probably due to this reason. From four of these mutants no opaques could be recovered in their progeny. With the exception of these, the high lysine mutants in Table 2 can be placed into two classes, Class I opaque mutants were those which produced phenotypically normal plants

Table 2. Distribution of putative opaque mutants in terms of their relative increase or decrease in lysine content when compared to normal vitreous control sib seeds.

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

Table 3. Thirty-five putative opaque mutants with increase in lysine concentration of more than 50 percent.

Mutant No.	Puerto Rico origin	Percent protein	Lysine % protein	Lysine % sample	% Lysine increase	Germination	Phenotype: Kernel endosperm	Phenotype: Plant	Phenotype: fertile/sterile	Class	Trt.	Parent line	opaque:vit. ratio	Comments
P30 normal*	47058	11.4 12.6	3.50 1.78	.399 .224	178	poor	opaque vitreous	normal	sterile	II	2 ml DES	954114	164/426	
P31 normal	47058	10.8 11.2	3.48 1.95	.376 .218	172	none	opaque vitreous	--	--	II	2 ml DES	954114	251/722	
P43 normal	50038	16.7	2.51	.418	152	none	homozygous opaque	--	--	II	2 ml DES	954114	--	
P45 normal	50065	13.9 12.8	2.96 1.84	.410 .235	174	normal	opaque vitreous	grassy	--	II	1 ml DES	954114	106/463	
P46 normal	50065	14.5 14.6	3.26 1.84	.473 .269	175	normal	opaque vitreous	grassy	--	II	1 ml DES	954114	69/318	
P81 normal	53031	15.9 17.0	2.51 1.41	.399 .240	166	poor	opaque vitreous	normal	sterile	II	2 ml DES	954114	95/285	
P139 normal	40079	13.7 12.5	2.75 1.97	.370 .245	151	poor	opaque vitreous	normal	fertile	--	1 ml DES	954114	49/356	No opaques recovered in the progeny
P171 normal	37082	20.1 19.3	2.32 1.57	.465	153	poor	opaque vitreous	normal	sterile	II	1 ml DES	954114	27/87	
P597 normal	18103	16.3 13.4	2.58 1.79	.420 .240	175	normal	opaque vitreous	normal	fertile	--	1 ml DES	954104	67/576	No opaques recovered in the progeny
P629 normal	15073	16.8 16.0	3.45 1.75	.580 .280	207	none	opaque vitreous	--	fertile	II	1 ml DES	954104	90/606	
P720 normal	08006-1	18.5 18.1	3.16 1.74	.585 .315	185	normal	opaque vitreous	normal	fertile	I	1 ml DES	954114	Information not available	
P721 normal	08006-2	15.7 14.5	2.99 1.90	.470 .275	170	normal	opaque vitreous	normal	fertile	I	1 ml DES	954114	"	
P726 normal	08093-2	15.9 10.9	2.63 2.40	.417 .262	159	normal	opaque vitreous	normal	fertile	--	1 ml DES	954104	"	No opaques recovered in the progeny
P728 normal	09009-2	20.0 19.3	2.49 1.57	.498 .303	164	--	opaque vitreous	Not planted			1 ml DES	909114	"	
P750 normal	13002	17.2 16.5	4.93 2.29	.848 .378	224	none	opaque vitreous	--	--	II	1 ml DES	909114	"	
P829 normal	27097-1	18.0 16.2	2.43 1.78	.436 .287	152	poor	opaque vitreous	normal	fertile	--	1 ml DES	954104	"	No opaques recovered in the progeny

Table 3. (Continued).

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

Table 3. (Continued).

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

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

when grown in Lafayette or Puerto Rico in 1973. Class II mutants on the other hand produced abnormal plant growth ranging from chlorophyll deficiency through dwarf or stunted plants. An example of one of these abnormal plants grown from opaque mutant P46 is shown in the Figure 3. Plants from these opaque kernels seem to have lost apical dominance and produce numerous short tillers, but no flowers or seeds. Plants grown from normal sib seeds from the same head produced normal plants as seen in the row right of the grassy type (Figure 3), which suggests that the opaque mutant itself is responsible for the abnormal sporophytic effects. That is, the grassy phenotype is probably a pleiotropic effect of the mutation producing opaque seed, but close linkage cannot be excluded at this time. There are many examples of Class II mutants (Table 3) where there was no germination whereas normal plants were obtained from vitreous sib seed from the same head.

The Class I high lysine opaque mutants produced plants with normal phenotypes and since these are most useful agronomically they were studied further. M_4 seeds of P720 and P721 were screened for homozygous opaque seeds over the light box. Fourteen heads of P721 and one head of P720 were found to be homozygous opaque. Seeds of these 14 homozygous opaque heads from the 1973-74 Puerto Rico planting were analyzed for protein and lysine content. Seed from one homozygous opaque head of P720 was also analyzed (Table 4). The average whole grain lysine content of P721 opaque was 2.8 (expressed as percent of protein) at 17.5% protein relative to the normal sib average of 1.7% lysine (expressed as percent of protein) at 15.4% protein. The average whole grain lysine content of P720 opaque was 2.9% (expressed as percent of protein)

Figure 3

Comparison of P46 opaque mutant plants with normal sib.

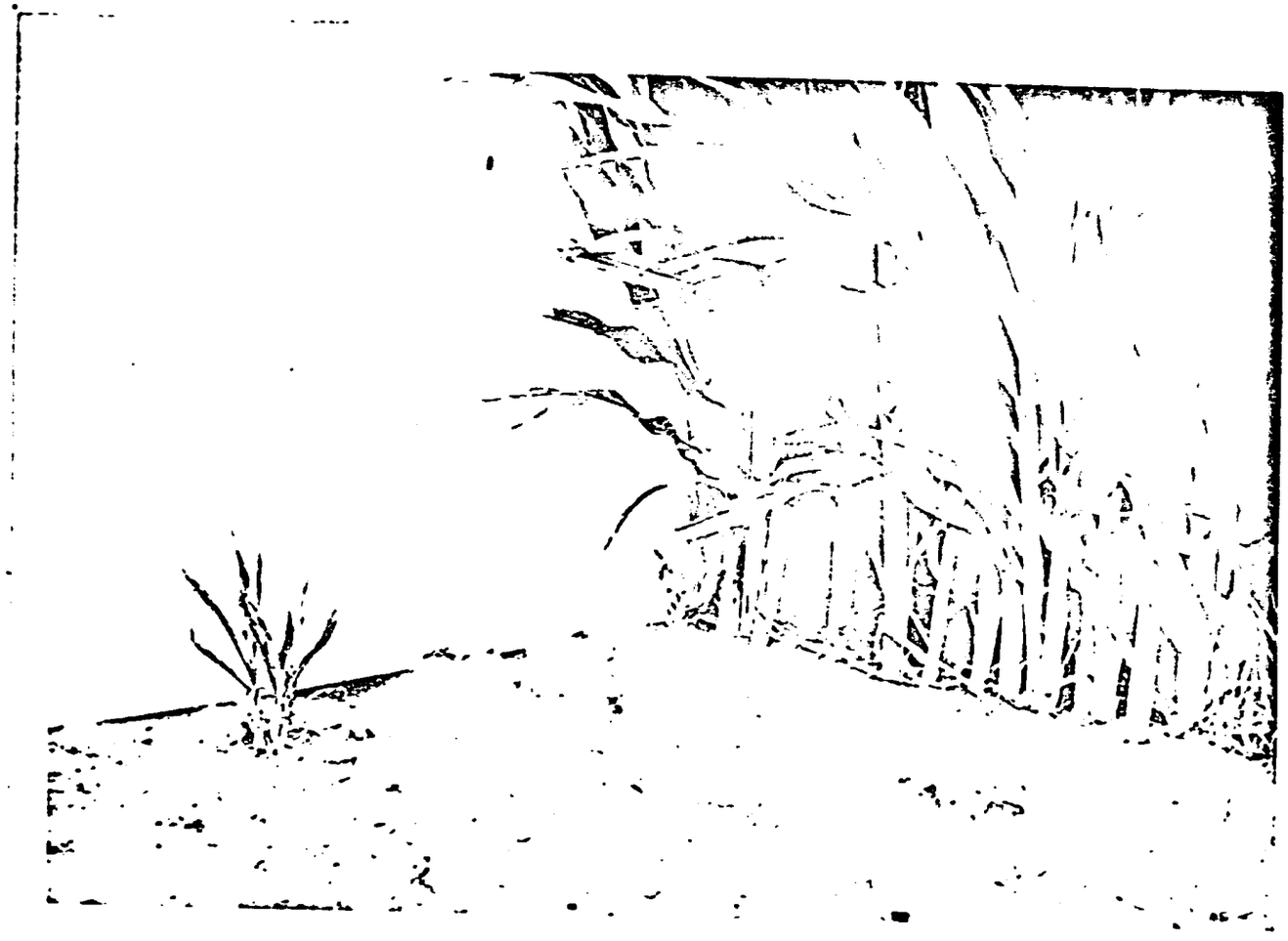

Figure 3

Table 4. Protein and lysine content of defatted whole kernels of P721 and P720 from Puerto Rico 1973-74 planting.

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

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

In 1974, P721 was increased at Lafayette and protein and lysine content of 14 sib families was analyzed (Table 5). The average lysine content was 3.09% of protein at an average protein content of 13.9%. The lysine concentration expressed as percent of sample was .432. Eight families from normal sibs were also increased in 1974 at Lafayette and analyzed for protein and lysine content (Table 5). The average lysine content was 2.09% of protein at an average of 12.09% protein which is about the average for lines in world sorghum collection (Srinivasan et al. 1972). The increase in lysine content over the normal sib is 60%.

As the seeds of P720 and P721 came originally from the same head row (see Table 3) it is presumed that they carry the same mutation for high lysine gene though the test for allelism has not been made.

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

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

Summary and Conclusions

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

Table 5. Protein and lysine content of defatted whole kernels of P721 and P720 from Lafayette 1974 planting.

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

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

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

SECTION II

INHERITANCE OF HIGH LYSINE P721 OPAQUE AND ITS AMINO ACID COMPOSITION

Materials and Methods

Inheritance Study

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

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

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

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

Chemical Evaluation

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

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

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

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

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

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

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

Results and Discussion

Inheritance of High Lysine Character

Phenotypic Segregation

Preliminary results of the inheritance study described here suggests that the high lysine character is probably simply inherited. Table 6 shows the distribution of vitreous and opaque kernels from three F_2 panicles grown from plants heterozygous for opaque mutant P721. Chi-square analysis indicates a reasonable fit to a 1:1 ratio in some cases but not in others. A semi-dominant mode of inheritance is suggested for P721 opaque as is true for floury-2 in corn where each successive dose of floury-2 in the endosperm adds an additional increment of lysine (Bates, 1966).

F_2 segregation data from three additional crosses is presented in Table 7. The female parents used as normal was CK60A, KS24A and PP3R $\underline{ms}_3$. The pollen parent was heterozygous for the opaque character. The F_1 seed obtained from the crosses was planted and F_2 seed from segregating heads was classified for opaque and vitreous endosperm by sectioning individual kernels. F_2 data from the cross with CK60A gave a satisfactory fit to 1:1 ratio. The other two crosses have more opaque than vitreous seeds. In fact, a ratio of 3 opaques to 1 normal will give a good fit in the second cross. For PP3R $\underline{ms}_3$, no simple ratio is obvious. All this variation could perhaps be explained by gene dosage effects in the triploid endosperm and modifiers from different genetic backgrounds.

Table 6. Classification of kernels from three branches of self-pollinated panicles segregating for opaque mutant P721.

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

* Based on 1:1 expected ratio.

Table 7. F_2 segregation and chi-square values for crosses involving P721 opaque.

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

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

Preliminary results of dosage series are shown in Table 8. There seems to be a dosage effect of the high lysine opaque gene in P721. Two doses of the opaque gene (+/o/o) increase the lysine content whereas one dose (+/+/o) has no effect. Results were preliminary since few seeds were available for analysis from hand emasculated crosses and the germ was not separated from the endosperm. There was also an untimely frost during the maturation of these seeds.

A preliminary test for allelism was performed with the high lysine mutants IS11758 and P721 opaque. All F_1 seeds appeared to be vitreous indicating the nonallelic nature of the two genes though there were a few opaque seeds perhaps because of the early frost. Table 9 shows the lysine and protein content of cross between P721 opaque and lines homozygous for hl gene and also a line considered as a normal check from the random mating Purdue Population PP20. There is no indication of an increase in lysine content which would be expected if these two mutant genes were allelic.

Amino Acid Composition of Defatted P721 Opaque and Normal Sib Kernels

Similar to the opaque-2 mutant of corn (Nelson, 1966) the effect of high lysine gene P721 opaque does not appear to affect the proteins produced in the embryo. Table 10 presents protein and lysine contents of defatted embryo of P721 opaque and normal sib and also the proportion of embryo and endosperm in these two classes of kernels. The lysine content in both classes of embryo was similar, though there was some increase in percent protein in the mutant class.

Table 8. Dosage effect of P721 opaque gene (Lysine g/100g whole kernel protein).

Martin $\underline{ms}_1$ used as normal parent	Martin $\underline{ms}_1$	Martin $\underline{ms}_1$ x P721 opaque	P721 opaque x Martin ms_1	P721 opaque
	+ + +	+ + o	+ o o	o o o
Lysine	2.37	2.32	2.90	3.24

Martin $\underline{ms}_3$ used as normal parent	Martin $\underline{ms}_3$	Martin $\underline{ms}_3$ x P721 opaque	P721 opaque x Martin ms_3	P721 opaque
Lysine	2.22	2.24	3.26	3.23

* o is abbreviation for the P721 opaque gene.

Table 9. Test for allelism performed for two high lysine mutants IS11758 and P721 opaque.

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

Table 10. Protein and lysine content of defatted embryo and endosperm and the proportion of embryo and endosperm.

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

* Pericarp was not removed from endosperm.

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

The amino acid composition of the defatted whole kernel and endosperm classes of P721 opaque and normal seeds is shown in Table 11. There is an increase in lysine and arginine concentration, and a decrease in glutamic acid, alanine and leucine concentration in opaque seeds relative to normal vitreous sib seeds. There is also an increase in the percent protein of opaque kernels. The shift in amino acid pattern of P721 opaque endosperm tissue is comparable to that reported for opaque-2 maize endosperm (Nelson, 1969) and hl endosperm in sorghum (Singh and Axtell, 1973).

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

Chemical Composition and Agronomic Traits of P721 Opaque

A comparison of P721 opaque characteristic with normal sibs and also with IS11758 and normal sorghum is shown in Table 12.

Table 11. Amino acid (g/100g protein) and protein content of defatted kernels of P721 opaque and normal sib sorghum lines.

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

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

Table 12. Comparison of high lysine line P721 opaque with various characters of IS11758, normal sib and normal sorghum.

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

* (Singh, 1973).

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

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

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

Summary and Conclusions

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

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

SECTION III

BIOLOGICAL VALUE OF HIGH LYSINE P721 OPAQUE MUTANT

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

Materials and Methods

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

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

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

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

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

Preparation of Diets

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

Experimental Design

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

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

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

Results and Discussion

All the diets were prepared at isonitrogenous level of about 10% protein. However, protein analysis data (Table 13) showed some difference in protein levels of various diets.

Data on the chemical composition of whole grain samples and composition of the diets as well as rat weight gain, feed efficiency ratio (FER) and protein efficiency ratio (PER), are given in Table 13. The average initial weight of weanling rats was 43.98 g. There was no significant difference in average initial weight between groups of rats placed on different diets. Weight gain on the P721 opaque diet was nearly three times higher than the normal sib weight gain. The PER values for P721 opaque rations was higher than the PER for normal sib rations but was lower than that for the casein diet. Weight gains at 7-day intervals through the 28-day feeding trial are shown in Figure 4.

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

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

Table 13. Biological values of isonitrogenous (10% protein) diets prepared with high lysine P721 opaque and normal sorghum lines in a 28-day rat feeding experiment.

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

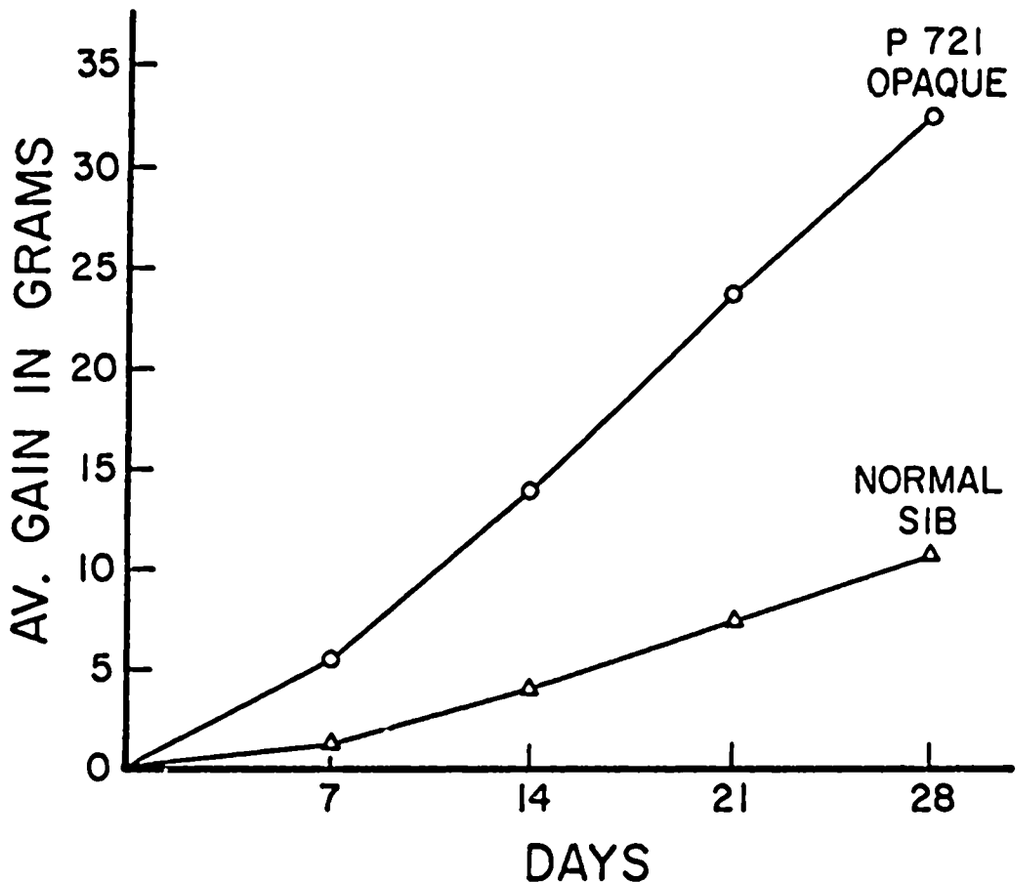

Figure 4. Average weight gain of rats fed on P721 opaque and normal sib rations at 10% protein level.

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

Summary and Conclusions

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

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

SECTION IV

[A] FRACTIONATION OF SOLUBLE PROTEINS OF HIGH LYSINE OPAQUE P721

[B] RAT FEEDING EXPERIMENT COMPARING (hl) SORGHUM IS11758 WITH P721 OPAQUE*

Subsection [A]

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

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

Materials and Methods

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

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

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

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

Results and Discussion

Table 14 gives the percent of endosperm, germ and pericarp expressed as percent of whole kernel. It is seen that germ of P721 opaque is about 11% larger than normal sib and average sorghum. The relative increase in germ size must be adding to the improvement of nutritional quality of P721 opaque kernel.

Table 15 gives the percentage distribution of nitrogen in five fractions obtained from the endosperm. The percent of soluble nitrogen is increased in P721 opaque in the first and fifth fractions and decreased in the second and third fractions when compared to normal sib endosperm.

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

Table 14. Percent distribution of endosperm, germ and pericarp of P721 opaque and normal.*

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

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

Table 15. Percent soluble nitrogen distribution in the P721 opaque and normal endosperm.*

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

* Average of two samples each.

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

Subsection [B]

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

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

Materials and Methods

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

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

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

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

Results and Discussion

Table 16 shows the chemical composition of whole grain samples and composition of rations as well as rat weight gain, feed efficiency ratio (FER), and protein efficiency ratio (PER). All grain rations were fed as isonitrogenous diets at approximately 10% protein. The average initial weight of weanling rats was 47.9 g. There was no significant difference in the average initial weight between groups of rats placed on different diets. Weight gain on the IS11758 and P721 opaque

Table 16. Biological values of isonitrogenous (10% protein) diets prepared with P721 opaque, IS11758 and normal sorghums in a 28-day rat feeding trial.

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

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

Summary and Conclusions

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

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

SECTION V

COMPARISON OF NUTRITIONAL VALUE OF HIGH LYSINE GRAIN SORGHUMS P721 OPAQUE AND IS11758 WITH CHICKS*

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

Materials and Methods

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

Experimental Procedure

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

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

start of the second week the heaters were adjusted to 90° F, and this temperature was kept until the end of the experiment, when the birds were 18 days old. The room temperature was approximately 75° F. Each diet was fed to four replicate groups of seven chicks. No two replicates of the same treatment appeared in the same brooder or the same deck level of another brooder. Feed and water were provided ad libitum during the 18-day study. Normal sorghum (diets 1 and 2, Table 17) was compared on an isonitrogenous basis with high lysine sorghums (diets 3 and 4). Glucose monohydrate was added as the adjusting nutrient so that the diet would contain the proper amount of protein. In all treatments the level of protein in the diet was kept at 10.09%.

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

Results and Discussion

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

Table 17. Composition of diets and the effect of sorghum grains RS610, IS11758 and P721 opaque on weight gain and feed conversion of chicks.

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

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

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

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

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

Summary and Conclusions

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

SECTION VI

COMPARISON OF THE ENDOSPERM STRUCTURE OF P721 OPAQUE AND THE NORMAL SIB FOR PROTEIN BODIES UNDER LIGHT MICROSCOPE

Except for Rooney and Sullins (1973), and Clark (1975), isogenic lines of high lysine sorghum have not been compared for protein bodies microscopically. It was first suggested by Seckinger and Wolf (1973), for sorghum, that an indirect measure of lysine content was by correlation between the size of protein bodies containing prolamine. This study was therefore undertaken to compare high lysine sorghum P721 opaque endosperm with normal sib which has an average amount of lysine to see if there were any apparent morphological differences in protein bodies between the two endosperms.

Materials and Methods

Five opaque and five normal seeds of P721 were obtained from five different sorghum heads heterozygous for the opaque character. These heterozygous heads were from the study 443 planted at Purdue Agronomy Farm, Lafayette in 1974.

Observations of endosperm protein were made on destarched sections as described by Wolf and Khoo (1970). Four micron thick sections were obtained from the same general area of the seeds. Sections were stained with iodine vapors and the cover slip mounted in a dilute solution of

I-KI and photographed through a microscope equipped with Nomarski differential interference contrast light system. The microphotographs were taken so that the aleurone layer was towards the top of the picture.

Results and Discussion

Figure 5 shows destarched cross section of the endosperm of P721 normal sib, which is almost all vitreous. There is more protein in the subaleurone layer as is seen by the larger area and intensity of stain than towards the center of the kernel. Seckinger and Wolf (1973) found that the thickness of the subaleurone layer is a variety characteristic and is related to total endosperm protein content. The endosperm protein content of the normal sib is 11.7%.

The microphotograph in Figure 6 under higher magnification shows that the protein bodies have a beaded appearance. Large areas of subaleurone protein appear as a mass of microscopic granules in the protein matrix. Wolf and Khoo (1970) reported that protein bodies in sorghum endosperm are similar to maize but larger and more numerous. Duvick earlier in 1961 had reported that protein bodies in maize decrease in size from the aleurone layer inwards until they are no longer visible in the center of the endosperm. This observation seems to be true for sorghum protein bodies in the normal sib of P721.

Increased magnification under oil immersion (Figure 7) shows protein bodies quite clearly as spherical granules. These bodies are soluble in hot aqueous ethanol which is a good solvent for extracting prolamine from sorghum (Jones and Beckwith, 1970). This confirms that protein bodies are a major site of the prolamine fraction in sorghum.

Figure 5

Protein bodies in the endosperm section of P721 normal sib. Interference contrast (Nomarski) X 368 (Section 4μ thick destarched with alpha-amylase. Voids left by starch after digestion with alpha-amylase. Stained with iodine vapor. Protein shows two components, a matrix and granular bodies. Al, aleurone layer; M, matrix; Pb, protein bodies.)

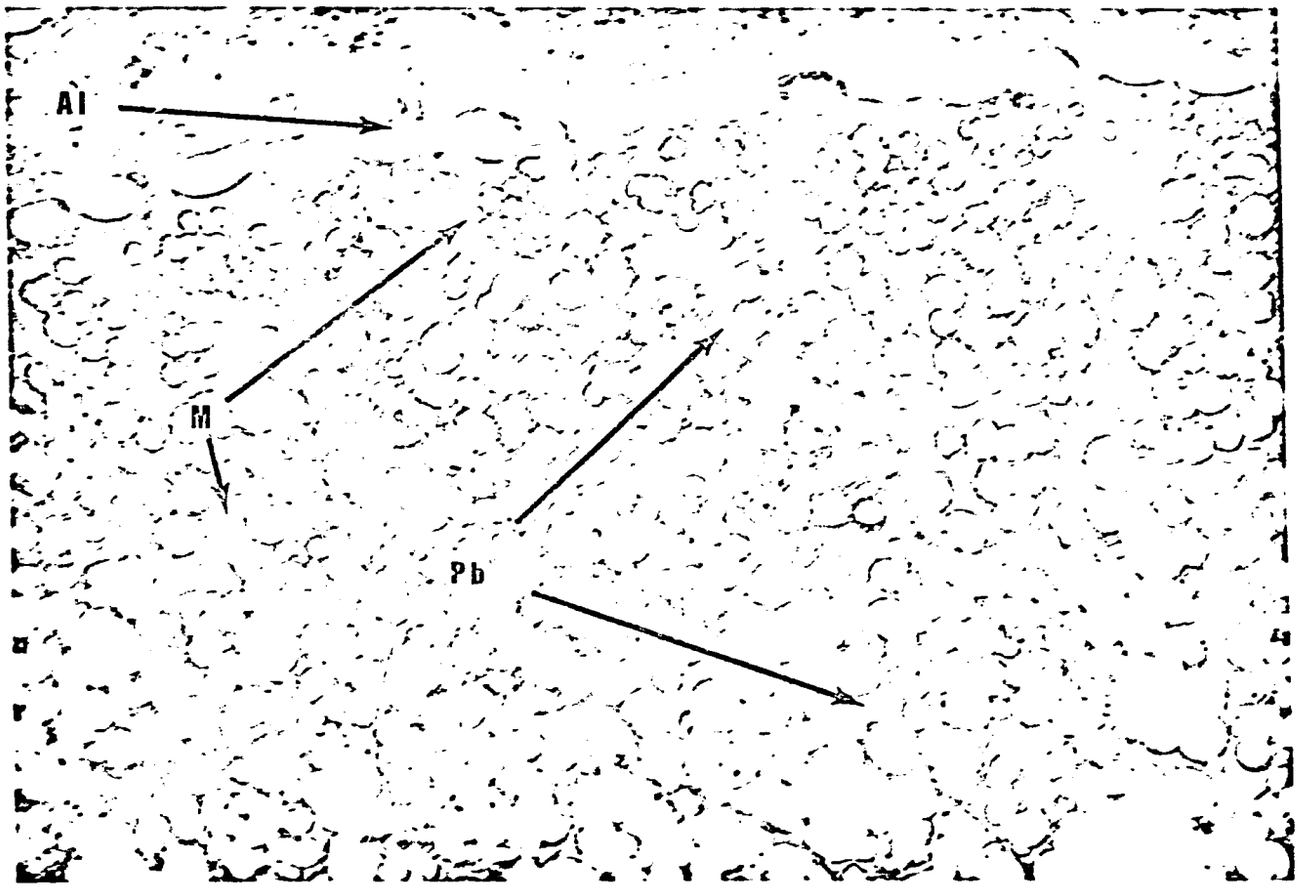

Figure 5

Figure 6

Protein bodies in the endosperm section of P721 normal sib. Interference contrast (Nomarski)X 920 (The aleurone layer is towards the top of the picture. Protein bodies giving a beadlike appearance. Endosperm section 4μ thick. Stained with iodine vapor after destarching with alpha-amylase. Pb, protein bodies.)

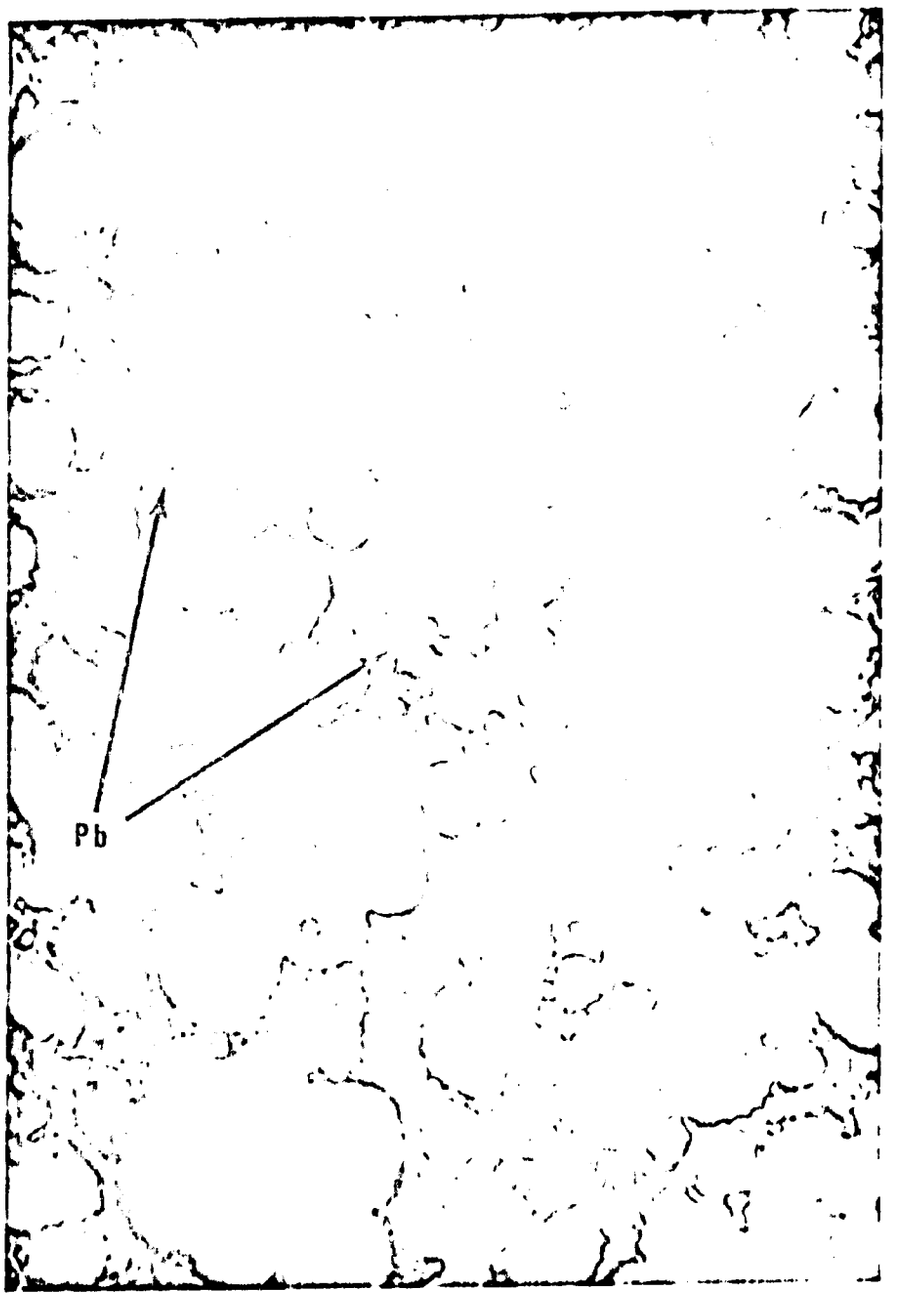

Figure 6

Figure 7

Protein bodies in P721 normal sib. Interference contrast (Nomarski) X 2,300 (The aleurone layer is towards the top of the picture. Section 4 μ thick. Stained with iodine vapor after destarching with alpha-amylase. Pb, protein bodies.)

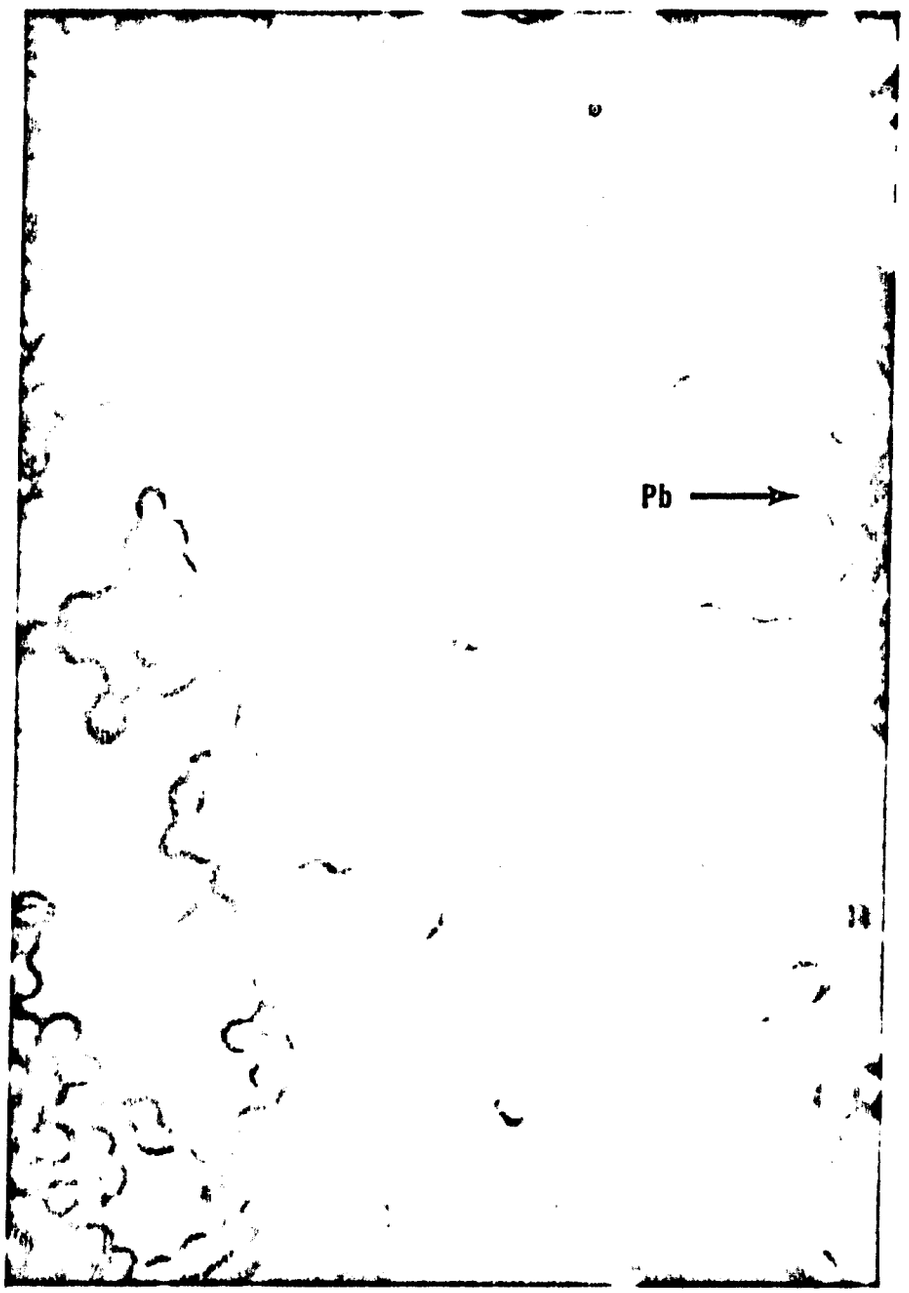

Figure 7

The prolamine fraction has the lowest lysine content of all protein fractions (Jones and Beckwith, 1970).

If there is an increase in lysine content then the number of protein bodies should be reduced. This can be confirmed by looking at the cross section of the endosperm of P721 opaque as shown in Figure 8, which is almost all floury. The protein network is loose and the cell size is bigger than that seen in Figure 5. The subaleurone layer is not as thick and there are no visible protein bodies (Figure 9) under a light microscope even under oil (Figure 10) in the subaleurone layer in contrast to normal sib which was tightly packed with them. There are no visible protein bodies elsewhere either in the endosperm. These observations on P721 opaque confirms the observations of Seckinger et al. 1973 that an indirect measure of lysine content was a correlation between size of protein bodies with prolamine content. In high lysine P721 opaque there are no protein bodies visible under light microscope or their size is so small that they remain undetected.

Summary and Conclusions

Observation under light microscope of the destarched endosperm sections of P721 opaque show that there are no visible protein bodies. As protein bodies are the major sites of alcohol soluble proteins which has the lowest content of lysine, it is possible that with the high lysine content the number and size of protein bodies have been reduced or eliminated beyond the resolving power of light microscope. The absence of protein bodies in P721 opaque is a definite morphological difference when compared to normal sib.

Figure 8

Protein matrix structure in the endosperm section of P721 opaque. Interference contrast (Nomarski) X 368 The aleurone layer is towards the top of the picture. Section 4 μ thick. Stained with iodine vapor after destarching with alpha-amylase. Granular protein bodies absent (compare with figure 5).

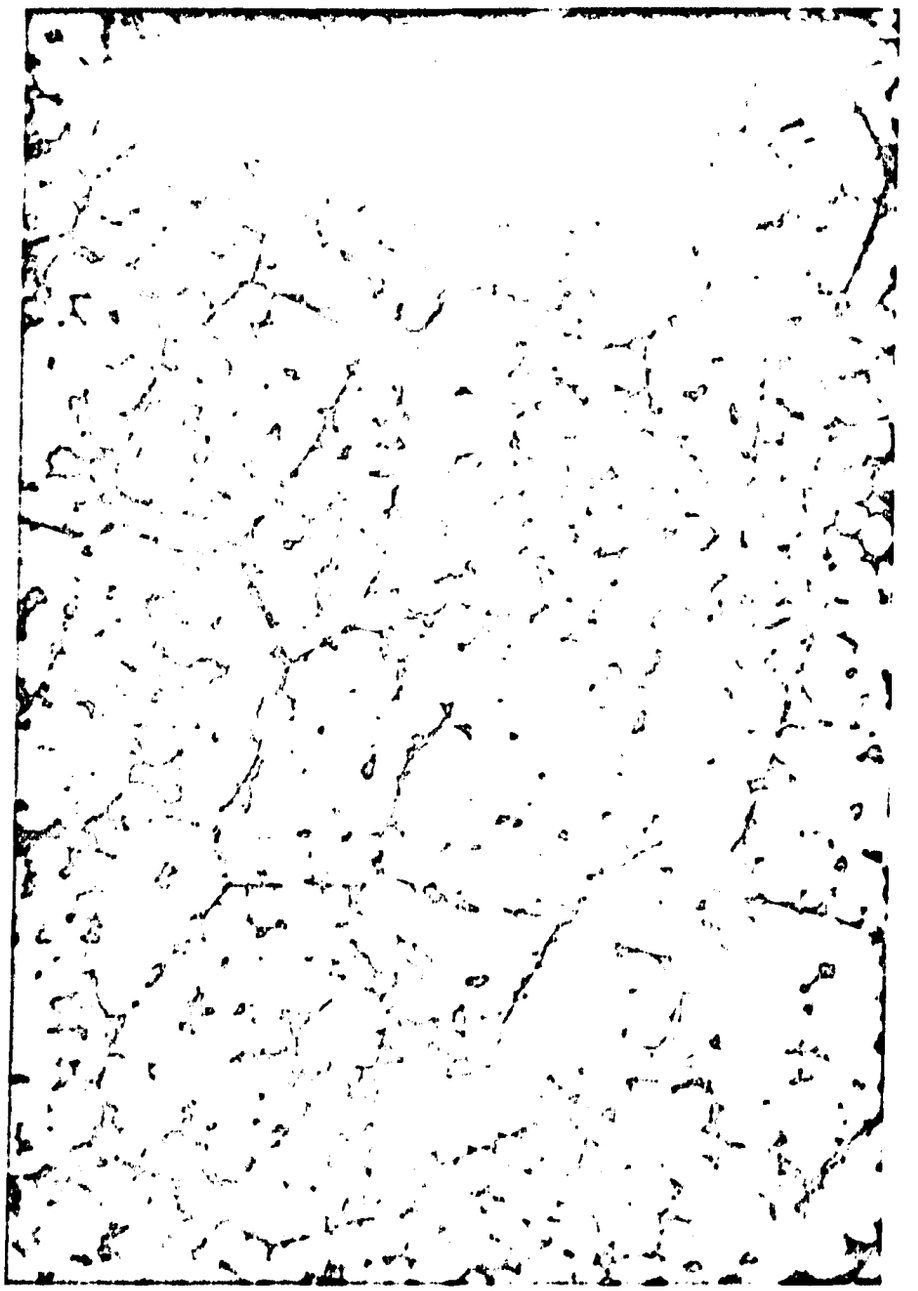

Figure 8

Figure 9

Protein matrix structure in the endosperm section of P721 opaque. Interference contrast (Nomarski) X 920 The aleurone layer is at the top of the picture. Granular protein bodies absent (compare with figure 6). Section 4μ thick stained with iodine vapor after destarching with alpha-amylase.

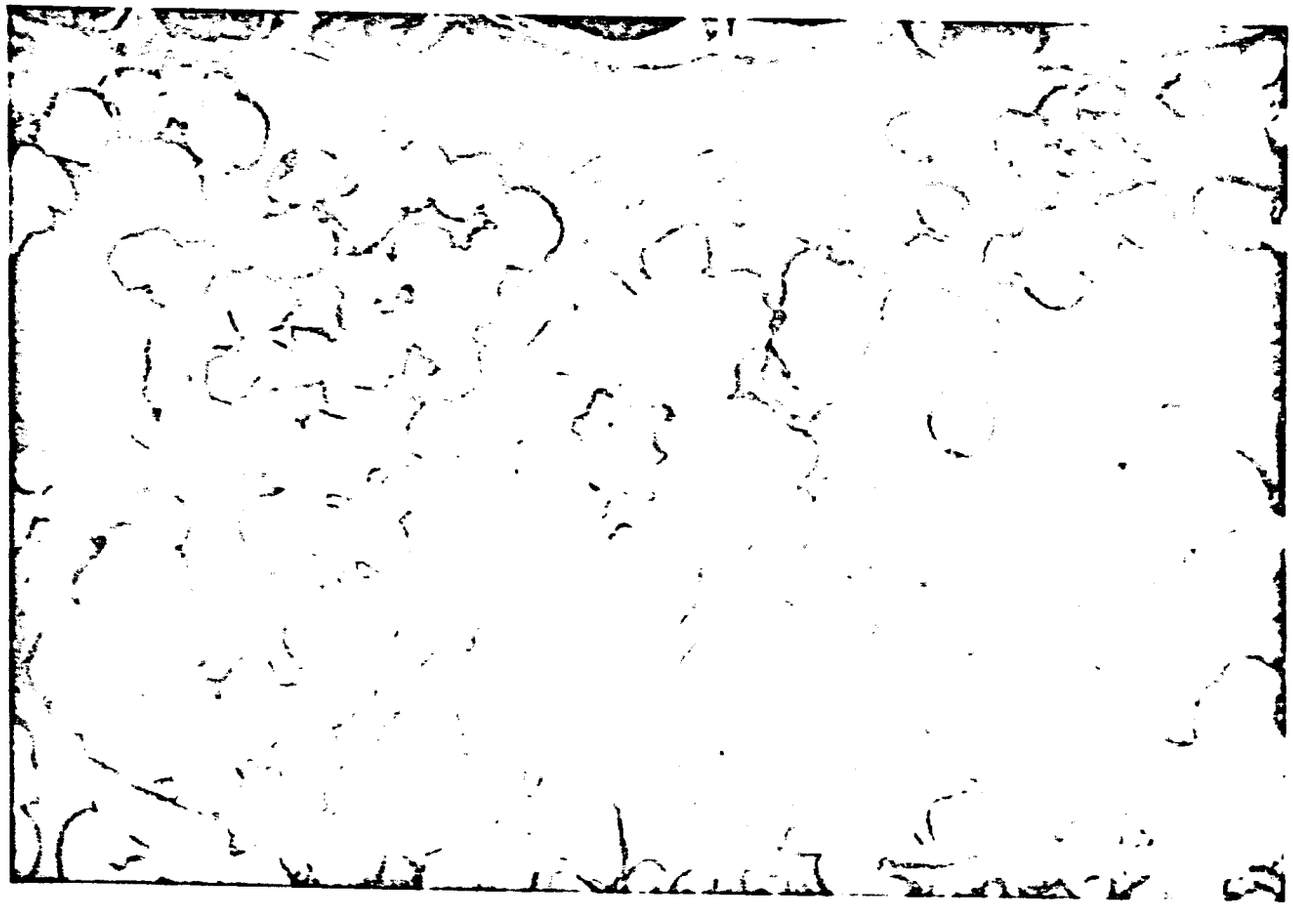

Figure 9

Figure 10

Protein matrix structure in the endosperm section of P721 opaque. Interference (Nomarski) X 2,300 The aleurone layer is towards the top. Granular protein bodies absent (compare with figure 7). Section 4 μ thick stained with iodine vapor after destarching with alpha-amylase.

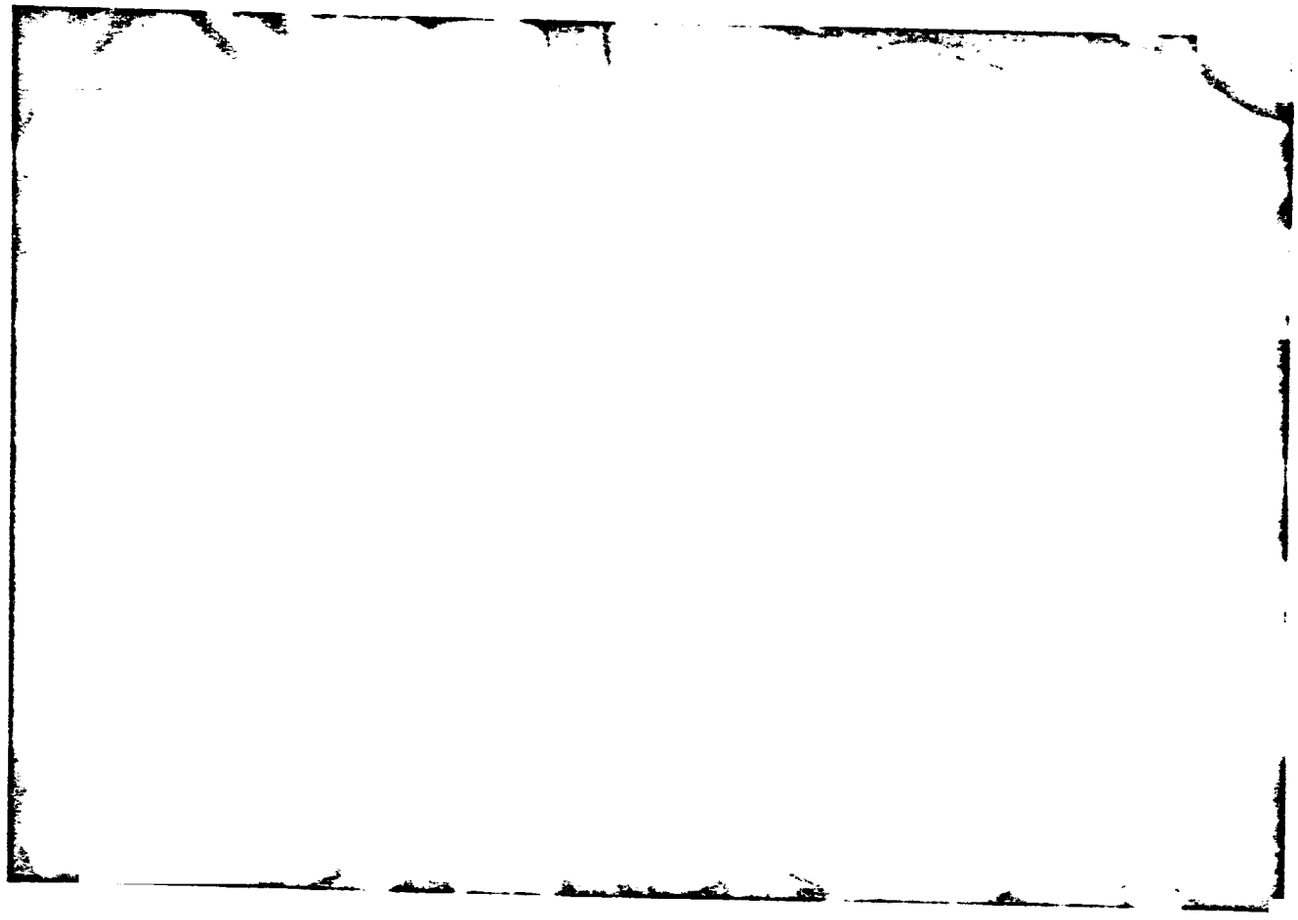

Figure 10

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APPENDIX

Table A1. Single analysis of defatted whole kernels of chemically induced putative opaque mutants in sorghum for protein and lysine content.

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)	Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P1-1	18.4	2.02	.372	P12-1	15.8	2.46	.389
-2	19.2	1.81	.347	-2	15.9	1.80	.285
P2-1	17.5	2.29	.401	P14-1	11.5	2.34	.268
-2	18.1	1.61	.290	-2	11.0	2.11	.231
P3-1	22.1	1.91	.421	P16-1	16.0	2.09	.333
-2	20.2	1.70	.342	-2	16.1	1.91	.307
P5-1	15.9	2.09	.331	P17-1	14.7	2.21	.325
-2	15.6	1.81	.281	-2	13.6	1.88	.256
P6-1	12.2	2.76	.337	P19-1	16.9	2.09	.352
-2	12.2	2.11	.256	-2	16.3	1.75	.284
P7-1	17.8	1.81	.322	P22-1	11.4	2.00	.228
-2	17.3	1.74	.301	-2	9.7	2.48	.241
P8-1	20.0	2.11	.422	P24-1	16.0	2.65	.424
-2	19.0	1.68	.319	-2	16.7	1.75	.291
P9-1	11.7	2.29	.268	P25-1	17.8	2.06	.367
-2	11.9	2.19	.259	-2	16.7	1.58	.264
P11-1	15.5	2.44	.377	P26-1	16.7	1.90	.316
-2	14.6	1.90	.276	-2	16.8	1.69	.284

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)	Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P27-1	13.1	1.93	.253	P38-1	13.6	2.56	.347
-2	12.7	2.06	.262	-2	12.6	1.91	.241
P28	15.7	2.33	.366	P39	16.6	2.27	.377
P30-1	11.4	3.50	.399	P40-1	8.8	2.54	.223
-2	12.6	1.78	.224	-2	9.3	2.13	.198
P31-1	10.8	3.48	.376	P41-1	8.0	2.57	.206
-2	11.2	1.95	.218	-2	9.2	2.12	.195
P32-1	13.1	1.84	.241	P42-1	10.2	2.59	.263
-2	12.6	1.83	.231	-2	10.0	2.31	.231
P33	10.5	2.13	.224	P43	16.7	2.51	.418
P34-1	11.1	2.40	.265	P44-1	13.1	1.74	.228
-2	10.8	2.31	.249	-2	12.5	1.84	.230
P35-1	13.1	1.97	.257	P45-1	13.9	2.96	.410
-2	12.3	2.09	.256	-2	12.8	1.84	.235
P36-1	12.0	2.11	.252	P46-1	14.5	3.26	.473
-2	11.6	1.96	.226	-2	14.6	1.84	.235
P37-1	10.9	2.28	.248	P47-1	13.4	1.87	.250
-2	11.3	1.93	.217	-2	14.0	1.89	.265

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P48-1	13.4	2.32	.311
-2	12.6	1.82	.228
P49-1	18.2	2.31	.419
-2	18.9	1.76	.333
P50-1	11.5	2.02	.231
-2	10.4	1.89	.197
P56-1	13.7	2.01	.275
-2	14.3	1.94	.276
P57-1	11.3	2.50	.282
-2	11.2	1.93	.215
P59-1	13.9	2.34	.324
-2	12.6	1.90	.238
P62	14.8	2.07	.305
P67-1	11.9	2.13	.252
-2	11.2	1.80	.202
P70-1	9.9	1.91	.189
-2	8.8	2.24	.196
P71-1	10.0	2.34	.234
-2	10.6	1.91	.201
P72-1	10.5	2.35	.247
-2	10.7	2.20	.234
P73-1	10.7	2.05	.218
-2	12.4	1.91	.237
P75-1	8.7	2.75	.238
-2	8.5	2.37	.200
P77-1	17.7	2.25	.897
-2	18.2	1.69	.307
P81-1	15.9	2.51	.399
-2	17.0	1.41	.240
P87-1	16.7	2.26	.376
-2	16.8	1.87	.313
P89-1	12.1	2.35	.283
-2	12.1	1.93	.233
P91-1	15.8	2.08	.329
-2	15.0	1.81	.271
P103	10.1	2.72	.275
P110-1	17.8	1.95	.346
-2	17.1	1.88	.320

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P113-1	11.2	2.37	.264
-2	10.0	1.95	.195
P117	16.4	2.23	.366
P126-1	14.4	1.80	.258
-2	14.2	1.76	.250
P136-1	16.2	2.01	.326
-2	16.2	1.87	.303
P138-1	18.7	1.87	.350
-2	17.2	1.98	.340
P139-1	13.7	2.75	.370
-2	12.5	1.97	.245
P145-1	10.2	3.09	.314
-2	11.2	2.22	.249
P148-1	13.9	1.90	.263
-2	14.1	2.01	.282
P152-1	12.1	2.34	.282
-2	11.6	2.23	.259
P157-1	10.1	2.59	.261
-2	10.4	2.60	.269
P167-1	15.0	1.93	.289
-2	14.4	1.83	.263
P171-1	20.1	2.32	.465
-2	19.3	1.57	.303
P172-1	14.6	1.88	.273
-2	13.7	1.87	.255
P173-1	10.0	2.39	.239
-2	9.2	2.33	.213
P174-1	19.6	1.99	.390
-2	19.3	1.70	.327
P177-1	18.2	1.99	.361
-2	17.4	1.64	.284
P178-1	18.2	2.13	.388
-2	18.0	1.44	.258
P189-1	10.3	2.49	.255
-2	9.9	2.17	.215
P198-1	15.0	1.90	.285
-2	15.5	1.83	.284
P199	15.4	2.14	.329

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P209-1	17.8	2.11	.375
-2	17.3	1.93	.334
P215-1	16.1	1.93	.311
-2	14.4	1.91	.275
P225-1	11.9	2.43	.288
-2	11.2	2.05	.230
P232-1	13.0	2.39	.311
-2	14.1	1.79	.251
P235	15.7	2.44	.382
P238-1	10.6	2.25	.238
-2	10.7	2.11	.226
P240-1	16.8	1.79	.301
-2	17.0	1.87	.318
P245-1	11.9	2.00	.238
-2	11.7	1.88	.220
P247-1	8.9	2.52	.223
-2	10.8	2.07	.223
P249-1	11.3	2.10	.236
-2	11.4	2.10	.238
P252-1	13.2	2.12	.280
-2	12.8	1.74	.223
P255	12.0	2.14	.257
P266-1	12.3	2.39	.294
-2	12.6	1.57	.198
P267-1	13.4	1.93	.259
-2	12.5	1.92	.240
P268-1	11.9	2.35	.280
-2	11.7	2.03	.237
P273-1	15.3	2.14	.326
-2	14.4	2.05	.294
P274-1	12.5	2.36	.295
-2	11.6	2.17	.252
P285-1	9.2	2.43	.224
-2	10.4	2.05	.212
P286-1	14.7	2.00	.294
-2	14.3	2.04	.294
P287-1	13.1	2.27	.296
-2	13.6	2.24	.305

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)	Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P291-1	13.4	2.08	.279	P584-1	11.7	2.15	.251
-2	13.5	1.87	.249	-2	11.0	1.94	.212
P292-1	18.2	2.14	.281	P585-1	14.6	2.14	.311
-2	13.5	1.83	.246	-2	13.8	1.68	.232
P293-1	14.1	1.93	.271	P586-1	14.7	2.24	.328
-2	14.3	1.77	.252	-2	15.7	1.88	.294
P294-1	16.1	2.06	.332	P587-1	16.2	2.16	.350
-2	14.6	1.96	.285	-2	15.3	1.67	.255
P297-1	16.1	2.19	.352	P588-1	16.1	2.00	.322
-2	14.5	1.87	.270	-2	15.8	1.63	.257
P301-1	17.5	2.04	.357	P590-1	13.7	1.99	.273
-2	20.6	1.40	.287	-2	14.4	1.80	.258
P317-1	12.0	2.04	.245	P591-1	15.8	1.86	.294
-2	12.6	1.75	.220	-2	16.2	1.76	.284
P334-1	11.7	2.46	.288	P592-1	16.2	1.89	.305
-2	10.4	2.00	.208	-2	16.0	1.81	.290
P335-1	12.9	2.18	.280	P593-1	14.3	1.98	.282
-2	13.4	2.06	.275	-2	13.2	1.78	.235
P583-1	13.7	2.38	.325	P594-1	14.5	2.04	.296
-2	13.4	1.88	.252	-2	14.4	1.68	.242

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)	Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P595-1	11.6	1.98	.230	P605-1	16.5	1.72	.284
-2	11.2	1.85	.206	-2	16.5	1.54	.253
P596-1	15.1	1.72	.260	P606-1	15.9	1.77	.280
-2	15.1	1.64	.248	-2	15.1	1.64	.248
P597-1	16.3	2.58	.421	P607-1	14.8	2.17	.320
-2	13.4	1.79	.240	-2	14.1	1.94	.292
P598-1	14.4	1.99	.286	P608-1	12.7	2.08	.263
-2	14.9	1.77	.264	-2	12.0	1.99	.239
P599-1	15.0	2.00	.300	P609-1	13.9	2.36	.328
-2	14.2	1.79	.253	-2	13.1	1.93	.253
P600-1	16.8	1.92	.322	P610-1	15.3	2.03	.310
-2	16.5	1.67	.275	-2	13.9	2.08	.288
P601-1	16.0	2.15	.344	P611-1	14.6	1.93	.282
-2	16.0	1.89	.301	-2	20.8	1.39	.288
P602-1	16.1	2.07	.332	P612-1	13.6	2.18	.295
-2	15.1	1.93	.290	-2	12.0	2.13	.256
P603-1	14.7	1.86	.272	P613-1	15.9	2.04	.323
-2	16.5	1.65	.271	-2	15.6	1.87	.292
P604-1	15.8	2.02	.318	P614-1	16.0	1.92	.306
-2	14.7	1.91	.281	-2	15.5	1.76	.273

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P615-1	18.0	1.80	.324
-2	18.2	1.72	.312
P616-1	16.5	2.38	.393
-2	17.0	1.68	.286
P617-1	17.8	1.84	.327
-2	16.3	1.89	.308
P618-1	13.2	2.44	.321
-2	13.1	1.92	.251
P619-1	18.0	1.83	.328
-2	17.3	1.79	.310
P620-1	15.1	2.17	.328
-2	13.9	1.79	.249
P621-1	17.6	1.73	.303
-2	17.4	1.67	.290
P622-1	17.4	1.69	.293
-2	17.2	1.67	.286
P623-1	17.3	1.93	.334
-2	17.4	1.82	.317
P624-1	14.6	2.02	.295
-2	14.1	1.86	.261
P625-1	14.0	1.89	.265
-2	14.0	1.80	.252
P626-1	16.8	2.06	.346
-2	15.0	1.79	.268
P627-1	13.9	2.34	.324
-2	12.8	1.85	.237
P628-1	16.6	1.98	.329
-2	15.9	1.85	.293
P629-1	16.8	3.45	.580
-2	16.0	1.75	.280
P630-1	12.5	1.91	.239
-2	12.7	1.83	.231
P631-1	14.8	2.02	.299
-2	15.0	1.76	.264
P632-1	18.1	2.02	.366
-2	18.1	1.86	.337
P633-1	16.6	1.87	.309
-2	15.8	1.71	.269
P634-1	18.7	1.68	.313
-2	18.9	1.65	.312

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P635-1	16.6	2.06	.342
-2	15.7	1.75	.275
P636-1	16.1	2.05	.330
-2	15.8	1.85	.291
P637-1	16.1	2.00	.322
-2	15.6	1.72	.267
P638-1	14.7	1.91	.281
-2	14.9	1.82	.270
P639-1	15.6	2.27	.353
-2	15.6	1.73	.270
P640-1	11.6	2.02	.233
-2	10.8	2.16	.232
P641-1	14.1	2.10	.295
-2	14.2	1.86	.263
P642-1	16.2	1.92	.310
-2	16.0	1.82	.290
P643-1	16.2	2.07	.334
-2	16.0	1.58	.253
P644-1	12.7	2.08	.263
-2	12.9	1.90	.244
P645-1	13.7	1.93	.263
-2	14.0	1.71	.238
P646-1	14.3	2.36	.336
-2	13.2	1.88	.247
P647-1	15.8	2.51	.396
-2	15.8	1.77	.280
P648	15.3	1.98	.303
P649-1	13.2	2.05	.271
-2	14.2	1.85	.263
P650-1	17.5	2.07	.361
-2	16.8	1.80	.301
P651-1	10.5	2.14	.225
-2	11.3	2.00	.226
P652-1	14.4	1.92	.275
-2	15.2	1.77	.269
P653-1	14.9	2.34	.349
-2	14.6	1.77	.257
P654-1	15.3	2.27	.346
-2	14.2	1.89	.267

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P655-1	13.8	2.42	.334
-2	13.1	1.71	.224
P656	15.4	2.07	.319
P657-1	17.8	1.78	.317
-2	17.6	1.59	.280
P658-1	12.5	2.02	.252
-2	12.7	1.98	.250
P659-1	16.0	2.03	.325
-2	15.7	1.73	.272
P660-1	18.3	1.95	.357
-2	17.4	1.78	.310
P661-1	10.9	2.14	.232
-2	10.2	2.26	.230
P662-1	14.3	1.90	.272
-2	13.1	1.79	.233
P663-1	16.1	2.68	.430
-2	16.0	2.35	.376
P664-1	11.6	2.31	.268
-2	11.7	1.79	.208
P665-1	16.0	2.66	.426
-2	14.8	2.00	.296
P666-1	14.7	2.26	.331
-2	13.7	1.89	.259
P667-1	9.9	2.28	.226
-2	9.8	2.26	.220
P668-1	14.6	2.16	.314
-2	14.8	1.59	.234
P669-1	16.1	2.10	.337
-2	15.2	2.03	.308
P670-1	17.0	1.80	.306
-2	16.6	1.73	.286
P671-1	16.4	2.00	.328
-2	15.7	1.71	.267
P672-1	7.6	2.81	.213
-2	7.7	2.61	.201
P673-1	17.3	1.92	.331
-2	16.8	1.77	.296
P674-1	11.6	2.54	.295
-2	10.8	2.12	.229

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P675-1	13.5	1.98	.266
-2	12.7	1.92	.244
P676-1	11.9	2.06	.244
-2	10.3	2.20	.227
P677	13.6	2.26	.306
P679-1	17.0	2.14	.364
-2	15.4	1.79	.276
P680-1	14.3	2.53	.362
-2	12.7	1.92	.244
P681-1	13.4	2.07	.276
-2	13.7	1.84	.251
P682-1	13.9	2.28	.317
-2	13.9	2.11	.292
P683-1	15.1	1.88	.284
-2	15.3	1.68	.257
P700-1	12.4	2.39	.295
-2	11.4	2.08	.236
P701-1	16.8	2.29	.385
-2	16.7	1.58	.264
P702-1	15.2	2.60	.394
-2	15.0	1.80	.270
P703-1	14.5	2.06	.299
-2	15.3	1.74	.265
P704-1	13.7	2.23	.305
-2	14.8	1.69	.250
P705-1	14.0	2.24	.314
-2	13.2	1.91	.251
P706-1	14.1	2.29	.323
-2	13.5	1.90	.256
P708-1	12.4	2.20	.273
-2	13.9	2.02	.281
P711-1	13.7	2.39	.326
-2	13.0	1.93	.251
P713-1	18.6	2.10	.391
-2	18.3	1.68	.306
P714-1	15.5	2.21	.342
-2	14.7	1.92	.281
P715-1	11.4	2.25	.256
-2	11.7	2.36	.275

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)	Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P716-1	17.5	3.93	.688	P728-1	20.0	2.49	.498
-2	17.9	3.95	.707	-2	19.3	1.57	.303
P717-1	17.3	1.92	.331	P729-1	17.0	2.10	.357
-2	17.5	1.71	.298	-2	15.9	1.96	.312
P718	17.2	1.96	.336	P730-1	12.9	2.65	.342
				-2	11.3	2.05	.232
P719-1	16.4	2.01	.330				
-2	15.5	1.89	.293	P731	14.9	2.10	.313
P720-1	18.5	3.16	.585	P732-1	12.8	2.03	.260
-2	18.1	1.74	.315	-2	11.7	2.00	.234
P721-1	15.7	2.99	.470	P734-1	13.7	2.07	.283
-2	14.5	1.90	.275	-2	15.2	1.43	.216
P722-1	13.8	2.08	.286	P735-1	16.4	2.07	.338
-2	13.0	1.90	.247	-2	15.0	1.94	.291
P723-1	14.6	2.73	.398	P737-1	16.3	2.29	.372
-2	13.6	2.40	.325	-2	15.4	2.00	.308
P724-1	17.0	2.56	.434	P738-1	19.3	2.09	.402
-2	17.7	1.73	.305	-2	18.3	1.70	.310
P725-1	13.7	2.82	.385	P739-1	15.3	2.34	.357
-2	12.9	2.20	.284	-2	15.6	1.88	.292
P726-1	15.9	2.63	.417	P740-1	16.6	2.26	.374
-2	10.9	2.40	.262	-2	15.6	1.97	.306

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)	Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P742-1	17.4	1.78	.310	P754-1	17.4	1.92	.333
-2	16.9	1.66	.280	-2	18.4	1.93	.354
P743-1	16.7	2.45	.408	P755-1	15.9	2.14	.340
-2	15.9	1.75	.277	-2	14.9	1.85	.276
P745-1	14.9	2.09	.310	P756-1	16.7	2.50	.417
-2	14.9	1.90	.282	-2	16.6	2.07	.344
P746-1	15.7	2.22	.348	P757-1	17.3	2.29	.395
-2	15.1	1.88	.284	-2	16.3	1.78	.289
P747-1	13.2	1.95	.256	P759-1	12.5	2.21	.275
-2	12.9	1.96	.253	-2	12.4	1.98	.245
P748-1	13.2	1.85	.243	P760-1	16.5	2.14	.352
-2	13.9	2.25	.313	-2	15.1	1.78	.269
P749-1	12.3	2.79	.342	P761-1	14.2	2.27	.321
-2	12.5	1.95	.244	-2	13.6	2.16	.294
P750-1	17.2	4.93	.848	P762-1	15.4	1.91	.293
-2	16.5	2.29	.378	-2	15.5	1.88	.290
P752-1	11.9	1.86	.220	P763-1	17.3	1.92	.331
-2	14.1	1.85	.261	-2	17.4	1.78	.310
P753-1	16.3	2.25	.367	P764-1	18.4	2.12	.390
-2	15.8	2.18	.343	-2	17.9	1.81	.324

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P765-1	13.4	2.16	.288
-2	12.2	2.21	.270
P766-1	16.7	2.02	.336
-2	15.8	1.94	.306
P767	18.5	1.82	.337
P768-1	13.9	2.27	.315
-2	14.5	2.11	.306
P769-1	14.0	2.37	.332
-2	13.0	1.89	.246
P771-1	14.3	2.19	.312
-2	13.6	1.95	.264
P772-1	14.9	2.01	.298
-2	14.0	1.93	.269
P773-1	16.6	2.06	.342
-2	15.4	2.16	.333
P774-1	11.2	2.32	.260
-2	11.4	1.87	.212
P776-1	17.0	1.84	.313
-2	18.1	1.82	.328
P777-1	15.0	1.93	.289
-2	16.5	1.89	.312
P778-1	13.5	1.99	.269
-2	13.0	1.84	.238
P779-1	14.5	2.24	.325
-2	14.6	1.72	.250
P780-1	19.2	2.03	.390
-2	18.7	1.76	.328
P781-1	16.2	1.97	.318
-2	15.7	1.76	.275
P782-1	8.9	2.59	.230
-2	8.9	2.35	.208
P783-1	9.3	2.16	.201
-2	9.5	2.08	.198
P784-1	12.0	2.24	.269
-2	10.3	2.23	.230
P785-1	14.0	2.10	.294
-2	13.7	1.86	.255
P787-1	13.4	2.24	.300
-2	13.7	2.15	.294

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)	Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P788-1	11.3	2.35	.265	P800-1	15.0	1.96	.294
-2	12.3	1.93	.236	-2	15.2	1.93	.292
P790-1	18.9	2.35	.443	P801-1	14.1	2.19	.309
-2	18.9	1.58	.299	-2	13.3	2.62	.347
P791-1	17.0	2.55	.433	P802-1	15.5	2.05	.318
-2	15.4	2.00	.308	-2	15.3	1.89	.288
P792-1	10.8	2.28	.245	P803-1	15.9	2.10	.334
-2	10.9	2.12	.230	-2	15.3	1.86	.284
P793-1	13.8	2.13	.294	P804-1	16.1	1.99	.319
-2	13.6	1.84	.250	-2	15.8	1.87	.294
P794-1	13.7	1.98	.270	P805-1	13.4	1.85	.248
-2	13.4	1.83	.244	-2	13.3	1.80	.238
P795-1	15.0	1.99	.299	P806-1	16.8	1.83	.306
-2	16.7	1.65	.275	-2	15.4	1.67	.256
P796-1	14.5	2.24	.325	P807-1	17.5	2.29	.401
-2	15.4	1.64	.252	-2	15.6	1.92	.300
P797-1	13.9	2.29	.317	P811-1	11.0	2.25	.247
-2	14.5	2.82	.409	-2	9.8	2.29	.223
P799	19.6	1.37	.268	P812-1	15.4	1.94	.299
				-2	14.3	1.88	.269

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)	Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P813-1	15.2	1.99	.301	P823-1	18.3	2.04	.372
-2	16.3	1.58	.257	-2	17.7	1.63	.288
P814-1	14.3	2.36	.336	P824-1	14.2	1.78	.253
-2	14.3	1.85	.264	-2	15.4	1.75	.269
P815-1	14.1	2.00	.282	P826-1	13.8	1.93	.265
-2	11.4	2.15	.244	-2	14.2	1.81	.256
P816-1	12.7	2.29	.291	P827-1	11.4	2.33	.266
-2	12.2	1.90	.232	-2	10.5	2.20	.231
P817-1	14.8	1.93	.286	P828-1	13.8	1.93	.265
-2	15.0	1.85	.277	-2	15.0	1.65	.247
P818-1	14.6	1.77	.257	P829-1	18.0	2.43	.436
-2	14.0	1.89	.265	-2	16.2	1.78	.287
P819-1	16.5	2.46	.406	P830-1	12.4	2.26	.279
-2	14.5	1.89	.273	-2	10.6	2.10	.225
P820-1	15.5	1.94	.301	P831-1	20.8	2.46	.512
-2	17.3	1.68	.291	-2	19.7	1.49	.293
P821-1	15.1	1.95	.293	P832-1	13.9	2.15	.299
-2	14.4	1.77	.255	-2	13.5	1.95	.262
P822-1	19.3	2.23	.429	P833-1	19.7	1.89	.371
-2	18.3	1.68	.306	-2	18.2	1.75	.318

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P834-1	14.4	1.97	.284
-2	13.4	1.92	.256
P835-1	12.7	2.41	.305
-2	11.7	2.04	.239
P836-1	13.4	2.44	.327
-2	14.1	1.75	.247
P837-1	11.9	2.09	.249
-2	10.7	2.17	.231
P838-1	18.3	2.40	.438
-2	17.8	1.79	.319
P839-1	11.3	2.24	.252
-2	11.1	2.07	.230
P840-1	15.0	2.07	.310
-2	15.2	1.75	.266
P841-1	20.0	2.03	.406
-2	16.9	1.68	.284
P842-1	15.1	2.34	.352
-2	15.6	1.66	.259
P843-1	17.0	2.65	.450
-2	15.9	1.66	.264
P844-1	12.9	2.44	.315
-2	12.3	1.97	.241
P845-1	17.9	1.86	.333
-2	18.8	1.80	.337
P846-1	15.3	1.87	.285
-2	15.5	1.86	.287
P847-1	13.1	2.50	.327
-2	12.0	2.24	.269
P848-1	16.6	2.03	.337
-2	16.9	1.66	.280
P849-1	12.3	1.89	.231
-2	11.7	1.97	.229
P850-1	12.8	1.92	.246
-2	13.3	1.86	.246
P851-1	12.0	3.29	.395
-2	11.7	2.00	.234
P852-1	12.6	2.09	.262
-2	13.2	2.03	.268
P853-1	17.9	2.19	.391
-2	17.6	1.80	.317

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P854-1	15.5	2.29	.355
-2	14.5	1.99	.288
P855-1	17.3	2.24	.387
-2	17.4	1.83	.317
P856	10.9	2.68	.291
P857-1	9.8	2.18	.214
-2	10.1	2.21	.222
P858-1	10.5	2.33	.245
-2	11.3	2.07	.234
P859-1	13.7	2.39	.326
-2	14.7	2.01	.294
P860-1	12.5	2.53	.315
-2	12.7	2.24	.283
P861-1	10.6	2.50	.265
-2	10.0	2.30	.230
P862-1	14.6	2.07	.301
-2	14.0	1.79	.251
P863-1	14.7	4.58	.672
-2	12.5	1.96	.245
P864-1	16.1	2.62	.422
-2	16.2	1.66	.269
P865-1	17.5	2.74	.479
-2	16.8	1.68	.281
P866-1	12.3	2.66	.326
-2	11.4	1.76	.201
P867-1	11.7	3.88	.454
-2	11.3	2.25	.253
P868-1	18.0	2.33	.418
-2	17.5	1.50	.262
P869-1	12.5	2.64	.330
-2	12.2	2.14	.260
P870-1	17.5	1.75	.305
-2	17.7	1.26	.222
P871-1	16.9	2.00	.338
-2	15.2	1.76	.267
P872-1	12.7	2.28	.289
-2	12.0	2.13	.256
P873-1	18.4	1.61	.295
-2	16.8	1.75	.294

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)	Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P874-1	17.8	2.28	.406	P888-1	15.6	1.95	.303
-2	15.3	1.97	.300	-2	17.7	1.52	.268
P877-1	11.2	1.86	.207	P890-1	9.2	2.42	.223
-2	12.4	2.13	.263	-2	8.6	2.39	.205
P878-1	10.5	3.05	.320	P891-1	12.5	1.84	.230
-2	10.1	2.18	.219	-2	11.9	2.50	.297
P879-1	17.9	2.11	.378	P892-1	13.7	2.06	.281
-2	17.7	1.62	.287	-2	13.2	2.10	.276
P880-1	8.4	2.98	.249	P893-1	12.2	2.10	.255
-2	9.2	2.35	.215	-2	11.9	2.11	.250
P881-1	14.2	2.48	.351	P894-1	10.4	2.65	.276
-2	12.4	2.09	.258	-2	9.8	2.23	.218
P882-1	14.9	2.92	.435	P895-1	13.8	2.05	.283
-2	15.4	1.62	.248	-2	13.7	1.90	.259
P885-1	12.9	2.22	.285	P896-1	15.6	2.26	.352
-2	12.5	2.19	.274	-2	14.0	2.07	.290
P886-1	14.6	1.97	.288	P897-1	14.4	2.40	.346
-2	15.2	1.92	.292	-2	14.3	1.90	.272
P887-1	15.6	2.05	.320	P898-1	15.0	2.14	.321
-2	16.2	1.95	.316	-2	14.1	2.19	.309

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)	Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P899-1	19.0	2.64	.502	P913-1	10.0	2.29	.229
-2	18.1	1.53	.277	-2	10.0	2.14	.214
P902-1	12.2	2.65	.322	P914-1	11.0	2.19	.241
-2	10.9	2.17	.236	-2	11.3	2.13	.241
P903-1	10.8	2.42	.260	P915-1	12.1	2.26	.272
-2	10.5	2.09	.218	-2	10.8	2.26	.243
P904-1	8.4	2.69	.226	P916-1	16.7	2.33	.388
-2	7.7	2.86	.219	-2	17.5	1.57	.275
P905-1	16.8	2.10	.353	P918-1	13.4	2.69	.359
-2	15.2	1.93	.292	-2	14.0	1.92	.269
P906-1	19.4	2.12	.410	P920-1	14.2	1.96	.277
-2	20.1	1.64	.330	-2	15.6	1.86	.289
P908-1	14.1	4.82	.680	P921-1	11.4	2.10	.238
-2	11.0	2.06	.227	-2	10.9	1.84	.200
P910-1	18.2	2.02	.368	P922-1	18.9	2.19	.414
-2	18.8	1.84	.346	-2	16.8	2.04	.343
P911-1	10.7	2.51	.268	P923-1	13.7	2.07	.283
-2	9.6	2.19	.209	-2	18.3	1.47	.269
P912-1	8.8	2.97	.260	P925-1	18.4	2.02	.372
-2	8.7	2.47	.215	-2	18.4	1.53	.281

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)	Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P926-1	20.9	2.36	.492	P937-1	14.0	2.09	.293
-2	20.0	1.51	.302	-2	14.2	1.98	.280
P927-1	15.2	2.14	.324	P938-1	17.2	2.65	.456
-2	15.5	1.78	.276	-2	17.2	1.66	.285
P928-1	16.0	2.38	.381	P939-1	15.7	2.26	.355
-2	14.5	1.56	.225	-2	15.1	1.96	.296
P930-1	18.4	1.83	.337	P941-1	17.4	2.03	.352
-2	20.6	1.35	.277	-2	17.1	1.81	.309
P931-1	18.9	1.87	.352	P942-1	15.9	2.34	.371
-2	18.0	1.51	.272	-2	16.4	1.69	.276
P932-1	16.8	2.59	.434	P943-1	10.4	2.25	.234
-2	15.7	2.08	.326	-2	9.7	2.22	.214
P933-1	10.9	2.32	.253	P946-1	15.2	2.52	.382
-2	11.5	2.00	.230	-2	12.8	1.44	.183
P934-1	19.6	1.61	.315	P948-1	15.9	2.04	.323
-2	18.4	1.54	.282	-2	14.8	2.13	.314
P935-1	13.9	1.91	.264	P949-1	13.5	2.10	.283
-2	13.9	1.87	.260	-2	12.9	2.08	.267
P936-1	9.3	2.41	.223	P952-1	18.0	2.38	.427
-2	9.3	2.03	.189	-2	17.8	1.56	.278

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P954-1	12.9	2.63	.338
-2	11.2	2.05	.230
P955-1	17.6	2.12	.372
-2	17.6	1.57	.275
P956-1	14.5	2.21	.319
-2	14.8	1.63	.240
P957-1	16.3	2.19	.357
-2	15.1	1.86	.281
P958-1	18.3	1.67	.306
-2	18.0	1.53	.274
P959	15.1	2.36	.355
P960-1	16.8	2.17	.364
-2	16.7	1.68	.280
P961-1	15.4	1.76	.270
-2	12.9	2.42	.311
P962-1	15.4	2.40	.370
-2	14.9	1.87	.279
P964-1	18.0	2.10	.378
-2	18.9	1.63	.308
P966-1	14.3	2.64	.377
-2	14.9	1.75	.261
P968-1	15.2	2.33	.353
-2	15.9	1.73	.274
P973-1	13.3	2.21	.294
-2	13.2	2.01	.264
P974-1	10.3	2.54	.262
-2	11.0	2.04	.223
P979-1	17.3	2.19	.379
-2	17.5	1.69	.296
P981-1	12.2	2.32	.282
-2	12.1	2.13	.258
P984-1	19.0	2.11	.401
-2	17.8	1.93	.344
P987-1	15.8	1.86	.294
-2	16.0	1.80	.288
P989-1	13.4	2.46	.330
-2	13.2	2.08	.274
P990-1	13.0	2.12	.276
-2	11.9	2.15	.256

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)	Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P991-1	17.4	1.90	.331	P1011-1	16.8	2.28	.382
-2	18.3	1.54	.282	-2	16.3	1.82	.297
P994-1	14.9	3.12	.465	P1016-1	16.8	1.93	.323
-2	14.1	1.77	.249	-2	15.5	1.92	.298
P995-1	17.7	2.28	.403	P1017-1	19.0	2.14	.407
-2	15.3	1.85	.282	-2	19.1	1.50	.286
P996-1	15.3	2.11	.323	P1021-1	13.8	2.88	.396
-2	13.8	2.10	.290	-2	13.7	1.90	.259
P997-1	20.3	1.90	.386	P1022-1	15.8	2.42	.381
-2	19.2	1.70	.325	-2	14.5	1.90	.275
P1001-1	17.1	1.93	.330	P1023-1	10.1	2.61	.264
-2	17.7	1.61	.285	-2	9.3	2.29	.213
P1002-1	11.8	2.20	.260	P1024-1	16.0	2.06	.330
-2	10.7	2.07	.220	-2	13.7	1.88	.257
P1004-1	17.4	2.22	.385	P1025-1	13.7	2.31	.315
-2	18.4	1.76	.324	-2	14.0	1.79	.251
P1005-1	17.8	2.01	.358	P1027-1	12.5	2.35	.294
-2	16.7	1.65	.275	-2	12.5	2.02	.252
P1008-1	14.1	2.24	.316	P1032-1	15.2	2.52	.382
-2	12.6	1.85	.232	-2	15.5	1.98	.307

Table A1. (Continued).

Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)	Putative Mutant No.	% Protein	Lysine (g/100g P)	Lysine (% of sample)
P1033-1	12.7	2.49	.315				
-2	12.4	1.94	.240				
P1035-1	17.9	2.05	.367				
-2	17.2	1.66	.285				
P1036-1	16.3	1.65	.269				
-2	18.7	1.63	.305				
P1037-1	18.2	2.09	.379				
-2	16.8	1.79	.301				
P1038-1	18.3	2.37	.434				
-2	18.3	1.67	.306				
P1044-1	16.5	1.78	.294				
-2	18.2	1.61	.292				
P1046-1	19.6	1.77	.347				
-2	20.3	1.71	.346				
P1048-1	21.5	1.97	.423				
-2	19.2	1.69	.323				
P1049-1	13.2	2.41	.317				
-2	13.0	2.17	.281				