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Review Article

BREEDING OF SORGHUM FOR HIGH LYSINE IN THE SEED

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Abstract- Sorghum grains have good composition in both amino acid and protein, but there is limiting amount/composition of lysine. Both genetic and environmental factors affect the protein content of sorghum. In sorghum the variability is high, probably because the crop is grown under diverse agro-climatic conditions,which affect the grain composition. The two high-lysine Ethiopian sorghum varieties, IS 11758 and IS 11167are identified to overcome the problem of limiting amount of lysine in sorghum seed through hybridization or mutation. The average lysine content of those varieties is higher than that of normal sorghum which grows under similar environment. Even the PER values for high-lysine varieties is higher than the normal value for sorghum. We need more data to understand whether the high-lysine gene in sorghum is stable in a normal plump seed endosperm background. Another high-lysine mutant, P721, was reported to have 60% more lysine than normal sorghum. The high lysine of P721 resulted primarily from unusually high amounts of Lysine-rich gluten and low Lysine-poor prolamin. They observed that in all three of these high-lysine sorghum varieties the Lysine content of the germ was normal but the Lysine content of the endosperm was higher than in normal sorghum. For the rapid estimation of protein and lysine in large numbers of sorghum samples, the Technicon autoanalyser method and the dye-binding capacity method were found to be most suitable. The relationship between yield and lysine in sorghum was not very strong. Therefore, it seems possible to increase the lysine content without much affecting the yield.

Keywords- Sorghum, Breeding, Lysine, Amino-acid and Seed

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Introduction

Characteristics and uses of sorghum

Sorghum [*Sorghum bicolor* (L.) Moench] is a monocotyledon crop belonging to the family Gramineae. It is naturally self-pollinated short day plant with the degree of spontaneous cross pollination, in some cases, reaching up to 30% depending on panicle types [1]. The annual wild and domesticated sorghums are diploid (2n = 2x = 20) and are of tropical origin. It is widely adapted to regions lying between 40^0 N and 40^0 S of the equator [2]. Although sorghum is cultivated both in tropical and temperate climates, it is best known for its adaptation to the drought prone semi-aridtropical (SAT) regions of the world.

In Africa and the developing countries of Asia, sorghum grains are used mostly for human consumption. Traditionally these grains are used in such diverse food types as leavened and unleavened flat breads, porridges, steamed foods, and rice-like boiled products and in alcoholic as well as non-alcoholic beverages [3]. Sorghum flours can be substituted for up to 20% of the wheat flour in making leavened bread. Outside the developing world, sorghum grain is predominantly used as animal feed. The feed value of pearl millet grain is greater than that of sorghum and comparable with that of maize [4]. Sorghum and, to some extent, pearl millet have the potential for several alternative uses, for which technologies are better developed in sorghum than in pearl millet.

Sorghum grain is as nutritious as other cereal grains; contains about11% water, 340k/cal of energy, 11.6% protein, 73% carbohydrate and 3% fat by weight [5]. It serves as astaple food for more than 500 million people in the semi-aridtropics of Africa and Asia. It is also used for preparing traditional beverages.

Both the grain and the stove rare also used as animal feed. Industrial use of sorghum form making sugar, starch, syrup, alcohol and molasses is increasing. With the frequent and cyclical occurrence of drought and erratic rain-fall, it could be an insurance crop to the small scale source poor farmers constituting the majority of the rural farming community in Ethiopia [6].

Sorghum is the fifth leading cereal grain worldwide after wheat, rice, maize and barley with area coverage of about 42.70 million ha and total production of 56.96 million metric tons [7]. In Africa, the area under sorghum production is about 24.44 million ha and total production and average yield being 20.84 million metric tons and 0.85 ton/ha, respectively. Ethiopia is third largest sorghum producer in Africa next to Nigeria and Sudan. It is among the top four cereal crops tef, wheat, maize and sorghum in Ethiopia (CSA, 2010). It ranks 3rd in area cultivated to cereals and 2nd in total production. The area under sorghum production was estimated to be 1.25 million ha with a total production and average yield of 1.72 million tones and1.37tones/ha, respectively [8].

Description of sorghum

It is one of the most important coarse-grain cereals in the drier regions of the world. Sorghum is grown on 45 million ha annually and ranks fifth in global cereal hectare. Sorghum is relatively more widespread than pearl millet, being cultivated in 86 countries of the tropical, subtropical, and warm temperate regions of the world. India, China, Sudan, Ethiopia, Niger, Nigeria, Burkina Faso, Mali, the Central African Republic, Mexico, and the U.S.A. account for 75% of the global grain sorghum hectare[9].

Sorghum is a C4 species with high photosynthetic efficiency and dry matter

production ability, and yet the grain yields of the crops, largely under subsistence farming in much of semi-arid tropical Asia and Africa, are low: 700-900 kg·ha-1 for sorghum. Low soil fertility, rain-fed farming characterized by erratic and inadequate rainfall (annual precipitation as low as 600 mm for sorghum), negligible external inputs, continued use of mostly unimproved cultivars with a low harvest index (<20%), and the prevalence of diseases and insect pests are the major causes of low productivity. Under these conditions, yields of maize become too uncertain and sorghum stand out as the most reliable food cereals.

The cultivation of improved cultivars, especially hybrids, in environments better endowed with respect to soil fertility and moisture availability gives higher grain yields. For instance, the average grain yields of sorghum cultivars maturing in 100-120 days are as high as 3.4 t·ha-1 in Mexico, 4.1 t·ha-1 in China, and 4.3 t·ha-1 in the U.S.A. Sorghum is also useful forage crops, and their Stover is used for fodder, fencing, thatching, and fuel purposes. Stalks of juicy "sweet sorghum" have additional uses in syrup and alcohol production. However, genetic improvement of sorghum for these attributes has been limited. This paper will deal with grain quality traits that bear directly on breeding sorghum for high Lysine in the seed. Primarily with genetic enhancement aimed at increasing and stabilizing grain yields, but will also address those quality aspects on farmers' preferences. The objective of this paper is;-

- To review the types of gene actions involved in controlling lysine and related traits in sorghum;
- To review pertinent information/knowledge on how these genes increase the lysine content of sorghum seed; and
- Generally document the discoveries on amino acid composition of sorghum seed.

Main Body

Amino acid composition of Sorghum

Amino acids are key nitrogen containing compounds and protein constituents, and their metabolism is a fundamental process to plant growth and development. Amino acid and nitrogen metabolism studies are essential in order to improve our understanding of several distinct aspects of general plant metabolism [10]. Amino acid metabolism in plants is controlled by a complex regulatory network involving a large number of enzymes and intermediates [11]. Lysine, methionine, isoleucine and threonine are essential amino acids that are present in limiting amount in the seeds of many crop plants and are considered to be of great nutritional importance in animal feed stuffs and human food [12]. These essential amino acids are synthesized Lysine and threonine biosynthesis in sorghum seeds in plants via different branches of a pathway that commences with aspartate [13].

The quality of a protein is primarily a function of its essential amino acid composition. To assess the protein quality, Block and Mitchell (1946) introduced the concept of an amino acid or chemical score, in which the amount of the essential amino acid that is in greatest deficit is expressed as a percentage of the amount present in a standard or reference protein. Egg and human milk proteins, for their very high biological value, have been considered as reference standards [14]. Sorghum protein differed in their essential amino acid profile [Table-1]. However, the most common feature was that Lysine was always found to be the most limiting amino acid. The highest deficit of Lysine was in the protein of barnyard millet (chemical score 31), closely followed by little millet (chemical score 33). Sorghum protein, with a chemical score of 37, did not differ very much in quality from the proteins of barnyard and little millet.

Solubility of protein

Grain proteins are broadly classified into four fractions according to their solubility characteristics: albumin (water soluble), globulin (soluble in dilute salt solution), prolamin (soluble in alcohol) and glutelin (extractable in dilute alkali or acid solutions). In solubility fractionation studies with sorghum five protein fractions were obtained [Table-2]. The levels of albumin plus globulin were higher in pearl millet varieties than in sorghum, while amounts of the cross-linked prolamin, ß-prolamin, were higher in sorghum than in pearl millet.

Table-1 *Essential amino acid composition (mg/g) and chemical score of sorghum and millet proteins*

Grain	Isoleucine	Leucine	Lysine	Methi-onine	Cystine	Pheny-lalanina	Tyrosine	Threonine	Tryptophan	Valine	Chemical score
Sorghum	245	832	126	87	94	306	167	189	63	313	37
Pearl millet	256	598	214	154	148	301	203	241	122	345	63
Finger millet	275	594	181	194	163	325	-	263	191	413	52
Foxtail millet	475	1 044	138	175	-	419	-	194	61	431	41
Common millet	405	762	189	160	-	307	-	147	49	407	56
Little millet	416	679	114	142	-	297	-	212	35	379	33
Barnyard millet	288	725	106	133	175	362	150	231	63	388	
Kodo millet	188	419	188	94	-	375	213	194	38	238	

Sources: (FAO. 1995) [21]

Apart from a favorable essential amino acid profile, easy digestibility is an important attribute of a good-quality protein. Chemical score does not take into account the digestibility of protein or availability of amino acids. Biological methods based on measurement of growth and nitrogen retention assess the overall nutritional quality of the protein. These methods include determination of protein efficiency ratio (PER), net protein utilization (NPU), biological value (BV) and true protein digestibility (TDP).

Wide variability has been observed in the essential amino acid composition of sorghum protein [15]. Lysine content was reported to vary from 71 to 212 mg per gram of nitrogen and the corresponding chemical score varied from 21 to 62.

Among the cereal crops, sorghum [*Sorghum bicolor* (L.) Moench] is one of the most important sources of protein for millions of people (mainly in Africa) and for livestock worldwide. However, the protein quality of sorghum is low as a result of an imbalance in essential amino acids in the seed storage proteins in a manner similar to that of other cereal crops [16].

Enzymes facilitate formation of lysine

One major goal of plant science for many years has been the development of cereal crops with higher amounts of lysine and threonine in the seeds [17]. In order to achieve such a goal, the aspartate metabolic pathway has been investigated in detail, and important such a goal, the aspartate metabolic Path way has been investigated in detail, and important regulatory steps have been identified [18] [Fig-1]. Some key enzymes of the aspartate path way such as Aspartate-kinase (AK, EC2.7.2.4), homoserine dehydrogenase (HSDH, EC1.1.1.3), dihydro-dipicolinate synthase (EC4.1.2.52) and threonine synthase (EC4.2.99.2) have been isolated, purified and characterized in several plant Species [19]. Most have been shown to be present in different isoenzymic forms and under thecontrol of many genes [20].

AK catalyses the phosphorylation of aspartate to form b-aspartyl phosphate and up to three AK isoenzymes has been observed in plants, which are subject to feedback inhibition, either by lysine or by threonine. The lysine sensitive form of AK, which may also be synergistically feedback inhibited by a combination of lysine and S-adenosyl-methionine (SAM), is normally pre-dominant in plant tissues, accounting for approximately 50-70% of the total AK activity, whereas the threonine sensitive AK isoenzyme normally accounts for approximately 20% of the total AK activity.

In a branch of the pathway, aspartate semi-aldehyde is reduced to homoserine in a reaction catalyzed by the enzyme HSDH, which uses reduced nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) as a substrate. Two iso-enzymes, one sensitive to threonine feedback

inhibition and another resistant to threonine inhibition, have been observed in plants. The AK and HSDH iso-enzymes sensitive to threonine feedback inhibition have been shown to be part of a single bi-functional polypeptide. Lysine metabolism had not been previously investigated in sorghum. They have recently isolated lysine 2-oxoglutarate reductase (LOR, EC 1.5.1.8) and saccharopine dehydrogenase (EC 1.5.1.9), which are involved in lysine degradation, in sorghum seeds (Fornazier *et al*., 2005), but only preliminary results have been reported for AK and HSDH from sorghum seeds.

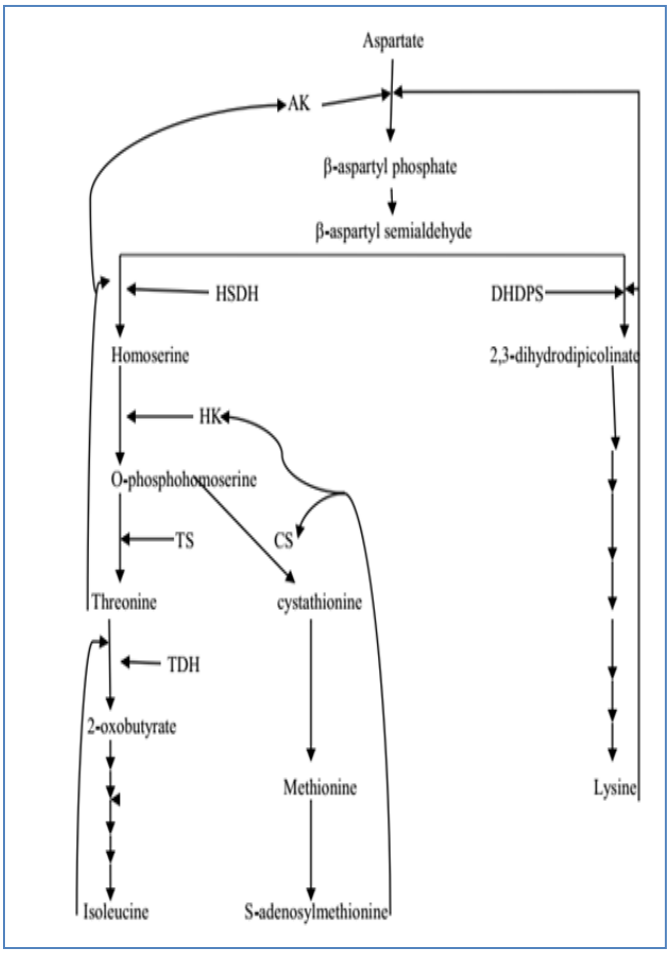

Fig-1 The aspartate metabolic path way leading to the synthesis of lysine, threonine, methionine and isoleucine. Enzymes: AK, aspartate kinase; CS, cystathionine γ-synthase; DHDPS, dihydrodipicolinate-synthase; HK, homoserine-kinase; HSDH, homoserine-dehydrogenase; TDH, threoninedehydratase; TS, threoninesynthase. Feedback inhibition and activation by the aminoacid send products are indicated.
Source;- (Hudson *et al*. 2005).

Protein distribution in high-lysine sorghum

The second major component of sorghum and millet grains is protein. Both genetic and environmental factors affect the protein content of sorghum and millets. In sorghum the variability is large, probably because the crop is grown under diverse agro-climatic conditions which affect the grain composition [21]. Fluctuations in the protein content of the grain are generally accompanied by changes in the amino acid composition of the protein [22]. Singh and Axtell (1973) identified two high-lysine Ethiopian sorghum varieties, IS 11758 and IS 11167. The average lysine content of the whole kernel of IS 11758 was 3.13 g per 100 g protein and the total protein content of the kernel was 17.2 percent. IS 11167 contained 3.33 g lysine per 100 g protein and 15.7 percent protein. Normal sorghum grown under similar conditions contained 12 percent protein and 2.1 g lysine per 100 g protein. Feeding trials in rates have shown higher PER values for high-lysine varieties (1.78 and 2.05 for IS 11758 and IS 11167, respectively) than for normal sorghum (PER 0.74 and 1.24) [23].

Table-2 *Distribution of protein fractions in sorghum and millet grains (percentage of total protein)*

Fraction	Sorghum		Pearl millet		Finger millet	
	Range	Mean	Range	Mean	Range	Mean
Albumin+globulin	17.1-17.8	17.4	22.6-26.6	25.0	17.3-27.6	22.4
Prolamin	5.2-8.4	6.4	22.8-31.7	28.4	24.6-36.2	32.3
Cross-linked prolamin	18.2-19 5	18.8	1.8-3.4	2.7	2.5-3.3	2.78
Glutelin-like	3.4-4.4	4.0	4.7-7.2	5.5	-	-
Glutelin	33.7-38.3	35.7	16.4-19.2	18.4	12.4-28.2	21.2
Residue	10.4-10.7	10.6	3.3-5.1	3.9	16.1-25.3	21.3
Total	91.2-94.0	92.9	78.6-87.5	83.9	74.7-83.9	78.7

Sources: (FAO, 1995).

Another high-lysine mutant, P721, was reported to have 60 percent more lysine than normal sorghum. Van Scoyoc, Ejeta and Axtell (1988) have demonstrated that the high lysine of P721 resulted primarily from unusually high amounts of Lysine-rich glutelin and low Lysine-poor prolamin. They observed that in all three of these high-lysine sorghum varieties the Lysine content of the germ was normal but the Lysine content of the endosperm was higher than in normal sorghum [24]. Ahuja, et al, 1970, using a modified extraction procedure, observed wide variations in the distribution pattern of protein fractions in the sorghum varieties [25]. Albumin ranged from 2 to 9 percent of total protein, while globulin ranged from 12.9 to 16 percent, prolamin from 27 to 43.1 percent and glutelin from 26.1 to 39.6 percent. Seasonal differences in the distribution pattern of protein fractions were reported: sorghum varieties grown in the Rabi (dry) season had less prolamin than when grown in other seasons. Studies on amino acid composition of the protein tractions showed that the albumin and globulin fractions contained high amounts of Lysine and tryptophan and in general were well balanced in their essential amino acid composition. On the other hand, the prolamin fraction was extremely poor in Lysine, arginine, histidine and tryptophan and contained high amounts of proline, glutamic acid and leucine. Present in the form of protein bodies, prolamin was found to be a predominant protein fraction directly associated with the protein content of the grain. Glutelin, the second major protein traction, is a structural component, the protein matrix in the peripheral and inner endosperm of the sorghum kernel. Both in vitro and in vivo studies have demonstrated wide variability in protein digestibility of sorghum varieties [26]. The rates digestibility of protein of sorghum varieties with intermediate and corneous endosperm texture was 70.3 and 74.5 percent, respectively. These values were lower than that observed for corn protein (78.5 percent). In certain sorghum varieties the presence of condensed polyphenols or tannins in the grains is another factor that adversely affects protein digestibility and amino acid availability [27]. In tannin-free sorghum varieties, observed that the protein digestibility was inversely correlated with total protein in the grain ($r = -0.548$, $p < 0.1$), total prolamin ($r = -0.627$, $p < 0.25$), cross-linked or ß-prolamin ($r = 0.647$, $p < 0.05$) and digestibility of ß-prolamin ($r = -0.727$, $p < 0.01$). The protein digestibility of sorghum grain was thus found to be extremely poor as compared to that for wheat (81 percent), maize (73 percent) and rice (66 percent). The improved nutritional quality in Opaque 2 maize is due to the decreased amount of prolamine (Zein) and the increased concentration of albumins, globulins, and glutelins, resulting in a larger amount of lysine in the whole kernel [28].

Methods of protein and lysine estimation in sorghum

Protein estimation

We needed simple, rapid, inexpensive, and reasonably accurate methods. Several methods are available for the estimation of proteins; some of the commonly used procedures include the micro or macro Kjeldahl method, which is still used as a standard for crude protein estimation, the biuret method, the Lowry method, estimation of ammonia using the Technicon auto-analyzer, and the near-infrared reflectance method .When the laboratory started to function in 1974, they tried the biuret procedure [29] for the estimation of protein in sorghum and obtained a correlation coefficient (r) of 0.91 with the micro Kjeldahl method. However, when they used this method for routine screening, the differences between biuret protein value and micro Kjeldahl value were especially large in the low protein range, as

shown in [Table-5]. Later they tried the Technicon auto-analyser (TAA) method and a very high correlation coefficient of 0.99 was obtained between the micro Kjeldahl and TAA protein values. During routine screening of samples using the TAA method, error percentage between TAA values and micro Kjeldahl values determined on random samples showed less than 2 percent variation over a wide range of protein content. Therefore, we are using the TAA method for the estimation of protein values in sorghum.

Table-3 *Chemical Composition and Seed Characteristics of Whole Grain Sample of High-Lysine and Normal Sorghum Lines*

Character	High-lysinelines		Normalsorghum
	IS11167	IS11758	
protein %	15.70	17.20	12.70
lysine, g/100 g protein	3.33	3.13	2.05
lysine, %of sample	0.52	0.54	0.26
Chemical composition			
oil %	5.81	6.61	3.32
Seed characteristics			
% germ	14.60	16.30	10.10
seed weight, g/100 seeds	2.78	2.45	2.75
Carbohydrate composition			
sucrose, % of sample	3.08	2.61	1.03
starch, % of sample	58.90	57.80	60.80

Source: R. Singh and J.D.(1973)

Table-4 *Nitrogen Distribution in the Whole Kernels of Normal and High-Lysine Sorghums (Percentage of Total Nitrogen)*

Fraction	Redlan	Redlan x IS 11758 F2 kernels.	IS117558	IS11167
I Saline	10.0	15.3	26.0(22.4)	25.3
II Isopropanol	15.7	26.4	10.3(13.7)	15.2
III Isopropanol+2-mercapto ethanol	31.3	26.5	19.6(20.2)	19.3
IV Borate buffer+ 2-mercapto ethanol	4.5	4.3	6.5(4.3)	4.5
V Borate buffer+ 2-mercapto ethanol + sodiumdodecyl sulfate	29.3	22.5	27.2(33.5)	29.5
Total nitrogen extracted (%)	90.8	95.0	89.6(94.1)	93.3
Protein %	13.53	13.0	18.5(15.6)	16.3
Lysine (g/100 g P)	1.56	1.85	3.27(3.1)	3.10

Source; - (Jambunathan *et al*., 1975)

Table-5 *Deviation of Biuret (B) Protein from Micro Kjeldhal (MKJ) Protein Values in Sorghum Samples*

Protein%	Class%	No. of samples	Deviation of B from MKJ (%)
5 - 5.9	5	12	- 22.7
6 - 6.9	6	44	- 17.9
7-7.9	7	69	-18.7
8 - 8.9	8	97	- 16.6
9 - 9.9	9	31	- 16.9
10 - 10.9	10	16	- 11.9
11 - 11.9	11	10	- 11.2
		279	- 16.6

Source;-(R.M. Johnson and L.E. Craney, 1971)

Lysine estimation:

For estimation of lysine content we used the dye-binding capacity (DBC) method [30]. This method is based on the principle that the basic amino acids (lysine, histidine, and arginine) react in an acid medium with a mono-sulfonic acid azo dye (acid orange 12) to form an insoluble complex, resulting in a decreased intensity of the solution. The proportion of the dye bound is directly related to the total basic amino acids in the sample, and the unbound dye can be conveniently measured colori-metrically as percent of transmission, expressed as Udy instrument reading (UIR). The UIR depends on the total quantity of protein in the sample and also on the basic amino acid concentrations in the protein. After protein determination by the TAA method, the weight of each of the samples was adjusted to contain 80 mg of protein, and the UIR value was obtained on the sample. The UIR value represents the total amount of basic amino acids in the sample. In order to speed up the analysis, and also to reduce the possible influence of sample size on UIR values, the procedure was slightly modified, as follows:

UIR values were taken on a one-gram weight of sample (instead of adjusting the sample weight to contain 80 mg of protein) and the readings (UIR) were divided by the percentage of protein (P) in the grain sample to obtain a ratio (UIR/P). This ratio was compared with the lysine value determined by ion exchange chromatography using amino acid analyzers. Using 58 sorghum samples, which had a range of 1.34 to 2.98 percent lysine and a UIR/P range of 2.32 to 4.57, they obtained a correlation coefficient of 0.93 between actual lysine concentration and UIR/P ratio. A regression equation was obtained using this correlation, and lysine values were predicted on routine samples by this method. They do check these estimated values by analyzing selected samples with the amino acid analyzer. We can now analyze, using the above procedures, about 140 samples for protein and lysine per day [31].

Breeding activities to improve lysine content in sorghum seed:

The discovery that the Opaque-2 gene in maize improves protein quality has stimulated great interest among breeders, nutritionists, and biochemists, and considerable progress has been made toward genetic improvement of plant protein quality in other cereals. Sorghum is the most important crops grown in the semi-arid tropics (SAT). If sorghum is to retain their place, and to increase, as major cereals for human food in the SAT where they are more productive and reliable than are other cereals, their grain quality is of paramount importance. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), created in July 1972 with headquarters in Hyderabad, India, has four main objectives. One of these is: "To serve as a world center to improve the genetic potential for grain yield and nutritional quality of sorghum, pearl millet, pigeon pea, chick pea and groundnut."A stable, high yield of the crop is one of the main objectives of ICRISAT, at the same time; theywere exploiting whatever latitude exists for other nutritional characteristics such as protein, lysine, starch types, oil, minerals, etc.Grain quality can perhaps be considered to consist of two main parts: (i) evident quality based on appearance, flavour, and cooking quality characteristics, and (ii) cryptic quality based on nutritional value. Recently there have been several reports on protein-calorie malnutrition, and criticisms have been leveled against the protein quality work in food grain samples. 9, 10 Studies conducted by the National Institute of Nutrition in Hyderabad, India, have shown that the primary deficiencies in the diet of people in India are mainly calories, vitamins, and minerals. More research is required to determine the extent to which vitamins and minerals are heritable before screening and selecting for these constituents.

Germplasm collection:

A major source for improving the nutritional quality of sorghum is the germplasm collection. The sorghum collection at ICRISAT exceeds 14,000 accessions. they have recently completed proximate and mineral analyses on 100 germplasm collections representing the following types: with lustre; with persistent sub-coat; completely corneous; almost corneous; intermediate; almost floury; completely floury; waxy endosperm, and with white, yellow, straw, light brown, brown, reddish brown, light red, red, grey, and purple seed coat colours [Tables-6,8]. They were grown on red soil at the ICRISAT farm in the 1976 Rabi season. The wide range obtained in minerals and trace elements indicates that it is possible for sorghum lines to contain various amounts of these elements. It is recognized that the mineral composition of grain is influenced by the environment, soil, and management conditions. However, this observation draws our attention to the importance of analyzing the advanced elite lines in the breeding program for all the possible chemical constituents so that any cultivar having a very low amount of any of the important constituents can be identified at an early stage.As in other cereals, lysine is the first limiting amino acid in sorghum. After screening more

than 9,000 accessions in the world germplasm collection, Singh and Axtell reported in 1973 that two sorghum lines of Ethiopian origin, IS 11167 and IS 11758, had exceptionally high lysine at relatively high levels of protein. Both lines were also high in oil percentage [Table-3]. The protein efficiency ratio (PER) values obtained with IS 11167 and IS 11758 were 1.78 and 2.06, respectively, as compared with the PER of 0.86 obtained for normal sorghum. Inheritance studies suggested that the increased amount of lysine in each line was controlled by a single recessive gene that could be easily transferred by standard plant breeding procedures.

Table-6 *Proximate Analysis of 100 Selected Sorghum Germ Plasm Samples*

	Range	Mean
Starch, %	55.6 - 75.2	70.8
Protein, %	10.6 - 18.5	14.1
Ether extract, %	2.1 - 7.1	3.3
Crude fibre, %	1.0 - 3.4	1.9
Ash, %	1.6 - 3.3	2.1
Sugar, %	0.8 - 4.2	1.3
Tannin, %	0.1 - 6.4	0.6
Lysine, g/100 g P*	1.37 - 3.39	1.7
100 seed wt. (g)	1.3 - 5.7	2.8
Grain hardness (kg)	1.8 - 10.2	6.5

Source; -(R. Jambunathan, 1980).

Selection: The optimal procedure for selection uses all the information available about each individual's breeding value, combined into an index of worth. It has been proposed that a better way to exploit genetic correlations with more heritable traits is to construct an index that combines information on all traits [31]. Moreover, multiple trait selection through the construction of selection indices can be used to avoid or minimize the declining level of negatively associated traits that can result from individual trait selection. Because genetic variance and heritability of grain yield are lower under stressful environments. Direct selection for yield per se is often not adequately effective [32]. The use of secondary traits positively associated with grain yield, genetically variable and highly heritable is worthwhile under such conditions [33]. BLUPs have become the method of choice in crop breeding to predict breeding values in the process of developing a selection index. It has also been used in forestry to compare the expected genetic gains and the level of relatedness of the selected population for the various selection criteria [34]. One of the functions of the Biochemistry and Common Laboratory Services Unit at ICRISAT is to assist breeders in selecting the desired cultivars and progenies with improved lysine concentration. Because they selected for vitreous plump grain, the screening method using a light-box for Opaque character could not be employed. Therefore, in the laboratory, they have evaluated several methods for the rapid and accurate estimation of protein and lysine. Progress that has been made in identifying suitable methodology to screen thousands of samples is described below. Based on laboratory values, ICRISAT breeders selected the best grain samples among progenies of random mating populations and crosses involving the Ethiopian hl gene. When the improvement of protein content and quality in the selected lines was followed year after year, it was observed that some of the selections showed a large fluctuation in protein levels [Table-7]. Also, we could not increase the frequency of occurrence of such plants very much, and this led them to question whether the high-lysine (hi) gene was stable in a normal (plump seed) endosperm background. It was clear that they needed more data before this program could be confined.

Table-7 *Variation in Protein Content of Sorghum Lines from Season to Season*

Entry	1974 Rabi			1975 Kharif			1975 Rabi		
	Protein%	UDY	N	Protein%	UDY	N	Protein%	UDY	N
79337 2	7.2	30.0	1	7.8	34	1	12.0	24.5	20
79339-3	7.1	30.5	1	7.9	28	1	13.5	24.5	6
79337-4	7.2	31.5	1	7.2	28	1	12.7	23.0	8
79751-4	7.6	30.0	1	7.8	30	1	15.1	25.5	6
79337-2	7.2	30 0	1	7.0	32	1	13.6	23.5	6
79339-3	7.1	30.5	1	7.5	31.5	1	12.0	23.0	4

Source; - (R. Jambunathan, 1980).

UDY-UIR per 80 mg protein. N-number of entries used to calculate the mean. Kharif-monsoon season, from June to October. Rabi-winter season, from October to March. Poly-phenolic compounds, also known as tannins, present in the grain of some sorghum cultivars substantially reduce the big-availability of protein and other nutrients, which indirectly has a major negative effect on the nutritional quality of grain sorghum. At the same time, many researchers have presented data supporting "bird-resistant" qualities associated with the brown (high tannin) sorghum in areas where bird damage is severed Weathering, deterioration of seed quality due to weather conditions, including pre-harvest seed germination, is reportedly less serious in high-tannin sorghums. More information needs to be obtained to understand the role of tannin in bird- and weather-resistance of sorghum grains. One of our current interests in sorghum is to determine the factors that affect/relate to consumer acceptance of products prepared from sorghum. Our target populations are sorghum and pearl millet consumers living in SAT regions. It is not uncommon to find farmers in indict growing a local cultivar in a small area for their own family use and another high yielding cultivar or hybrid in a larger area for selling on the market. Therefore, evident quality characteristics deserve an important consideration in a breeding program, and efforts need to be made to screen for any characteristics that might be associated with the preparation of food products. There are certain characteristics that are preferred by people and are associated with good food products. Some of the desired characteristics in sorghum are shown in (R. Jambunathan, 1980).

Table-8 *Mineral and Trace Element Analysis (mg/100g) of 100 Selected Sorghum Germ Plasm Samples*

	Range	Mean
Calcium*	5.5 - 53.1	26.2
Phosphorus	226.4 - 620.2	397.0
Magnesium	150.0 - 293.0	189.6
Sodium*	1.0 - 24.3	7.6
Potassium	323.8 - 801.4	509.1
Iron*	2.6 - 9.6	5.9
Copper	0.1 - 3.2	0.8
Zinc	1.9 - 5.7	3.3
Manganese	0.2 - 3.5	1.6

Source;- (R. Jambunathan, 1980).

Hybridization

Jambunathan *et al.*,1975, fractionated the protein of the two high-lysine (hi) sorghums and compared the distribution of proteins with that of F_2 kernels obtained from a cross between Redlan (a normal variety) and high-lysine lines. The distribution of proteins in these F_2 kernels and high-lysine sorghums, as shown in [Table-2], indicates that high-lysine sorghums have a lower percentage of alcohol-soluble fractions and higher percentages of saline-soluble fractions, (albumins and globulins) and the distribution pattern is similar to that of Opaque-2 maize. It is clear that the high-lysine F_2 sorghum kernels have protein distribution patterns similar to those of high-lysine sorghums.

Therefore, a systematic study is now being conducted by a research scholar on the variation of protein and lysine due to location, management, and environmental conditions, and on the stability of the high-lysine gene under these varying conditions. Preliminary results indicate that the crosses involving P721 are promising in that they give rise to a much higher frequency of high-lysine segregants. This study is still in progress.

As one of the ICRISAT's objectives is to improve the nutritional quality of sorghum, and the two high-lysine lines were available at the time when ICRISAT started to function, improvement of the nutritional quality of sorghum was included in the sorghum breeding program in 1973. Because the kernels of the high-lysine sorghums are floury in nature, are partially dented, and have low seed weight resulting in low yield potential, attempts were made to transfer the shrunken high-lysine (hi) grain of the Ethiopian cultivars to photoperiod-insensitive genotypes with plump, well-filled grains. Another chemically induced high-lysine mutant, P721, discovered at Purdue University, [35]. was also used in the crossing program at ICRISAT. The amino acid composition and fractionation data on P721 have been recently reported by Guiragossian *et al.*(1978) [36-40].

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Abbreviations:
PER: Protein Efficiency Ratio
NPU: Net Protein Utilization
BV: Biological Value
TPD: True Protein Digestibility

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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