INHERITANCE OF LYSINE CONTENT, AND ENVIRONMENTAL RESPONSES OF HIGH AND NORMAL LYSINE LINES OF *SORGHUM BICOLOR* (L) MOENCH IN THE SEMI-ARID TROPICS OF INDIA

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Kenneth Walker Riley

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FORWARD

This thesis is written in the paper style, specified in the 1976 Plant Science Thesis Preparation Guide. It contains four manuscripts numbered 1 to 4.

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ABSTRACT

One of the goals of sorghum breeding at the International Centre for Crops Research in the Semi-Arid Tropics (ICRISAT) has been to incorporate, into agriculturally useful varieties, the improved nutritional quality of the high lysine mutant P-721 and of Ethiopian lines containing the *hl* gene. Limited success in approaching this objective prompted investigation of the role of some genetic and environmental factors which might impede achievement of this goal.

Estimation of lysine values through the Udy dye binding capacity to protein ratio, which had been used in the selection procedure, related reasonably well to lysine values from amino acid analysis. However, of the lines which had been developed from material heterozygous for the *hl* gene, selected simultaneously for plump kernels and high lysine, were found to have retained the *hl* gene. The *hl* gene appeared to control both the high lysine character and a dented appearance of the kernels. In two lines, improved lysine levels were conditioned by two independent recessive genes. These genes also controlled a bulgy appearance in the endosperm.

A factorial analysis of sources of environmental variation showed that levels of fertilizer nitrogen, soil type, season, and management levels had important effects on levels of protein, lysine and grain yield. Substantial interaction effects among the sources were revealed.

Stability analysis revealed that the responses of the high lysine sources were different from each other and from those of normal lysine lines. Under low soil N conditions, the lysine level of P-721 was no higher than in normal sorghum. In higher fertility environments,

the nutritional advantage of P-721 became more pronounced. Changes in the proportion of prolamine protein in the endosperm of P-721 and a normal lysine line accounted for the different lysine response over environments.

The Ethiopian *hl* mutant, IS.11758, which possessed both higher protein and lysine levels than did P-721, maintained its nutritional superiority over environments. Both P-721 and IS.11758 produced low grain yields in low yielding environments. However, the yield of P-721 remained low, while that of IS.11758 rose rapidly with improvements in the environment. Although kernel weight of both mutants remained low in all environments, IS.11758 was able to compensate with very high numbers of kernels per head in the better environments.

Progeny from crosses with P-721 were found which maintained a nutritional advantage over the P-721 parent, but produced only a slight advantage over normal lines in low N environments. They resembled P-721 in producing floury endosperm, light kernels, and low grain yields. The *hl* gene in IS.11758 was a more useful source of improved protein quality. Benefits from breeding for wider adaptability could be expected in this line. In addition, a genetic component for high protein in IS.11758, shown to be independent of the *hl* gene, should provide a useful source of higher protein.

GENERAL INTRODUCTION

Cereals are an important source of protein in human diets, comprising 50 per cent of the dietary protein on a worldwide basis and about 70 per cent of the protein consumed in developing countries (Axtell, 1979; Kaul *et al.*, 1977). However, the composition of amino acids in cereal protein is less than ideal for the human, with all cereals primarily deficient in lysine (Juliano, 1972). Because of this imbalance, the average biological value (BV) of cereal proteins is only about 60 percent (Thielebein, 1969). Thus, the finding of genes that significantly increase the biological value of maize (Mertz, *et al.*, 1964; Nelson, *et al.*, 1965), barley (Munck *et al.*, 1971; Ingversen *et al.*, 1973) and sorghum (Singh and Axtell, 1973; Mohan, 1975) offers great promise in decreasing protein deficiency, providing that these genes can be incorporated into high yielding, adapted varieties, and made available to the people most affected by malnutrition.

During the past decade, the causes of malnutrition, and the role that cereals with improved protein quality might play in overcoming malnutrition, have been controversial topics. The existence of a "protein gap" between calculated needs and projected supplies (Altchul, 1972), has been questioned by an evaluation of more recent nutritional data which indicated that calories, rather than protein was the primary limiting nutrient in diets of those most prone to malnutrition (Ryan, 1977). However, diets with improved protein quality such as those with opaque-2 maize, have been shown to produce a nutritional improvement in young children (Singh and Jain, 1977).

A careful evaluation of these issues can help to determine the emphasis to be placed on improving protein quality in breeding programs, such as the cereal improvement programs at the International Crops Research Institute for the Semi Arid Tropics (ICRISAT). The high lysine breeding project was started in 1973 as a part of the sorghum improvement program at ICRISAT, which had the objective to "improve the yield and nutritional quality of sorghum...in the semi arid tropics." (ICRISAT, 1978.) A large consignment of breeding material from Purdue University was planted at ICRISAT during the 1973 cool, dry season. This material was composed mainly of selections from several populations which had been originally assembled in the U.S.A., or in Uganda, crossed with the high lysine (*hl*) mutants, IS. 11167 and IS. 11758, from Ethiopia.

Initial selection in the segregating material was for plants with good agronomic appearance, and plump, opaque seeds, which came from rows where other heads were segregating for the dented seed marker, characteristic of the *hl* gene. Selections were planted in the 1974 warm, wet (Kharif) season. A number of plants were intercrossed, and other heads were selected for good agronomic appearance.

These single head samples were then screened for high lysine. Screening procedures have changed somewhat over the years, but most selections have been screened with the dye binding capacity/protein ratio. Dye binding capacity (DBC) was determined on 1 g of ground sample (Udy, 1971), and protein percent (P) by the Technicon method (Mitcheson and Stowell, 1969). Samples which produced high DBC/P values were selected.

This method of selection, first on agronomic appearance followed by chemical screening for high DBC/P ratios, and intercrossing or chain crossing in progenies of the best selections, has been followed in this breeding work. Although the cut-off level for selection changed from season to season, the minimum level was a DBC/P ratio of 3.5 on a 1 g. sample basis. In each season, the breeding nurseries were given 100-200 kg N/ha, usually in two doses. They also received adequate moisture to prevent wilting, either as rain or as irrigation.

A number of promising early generation selections were made which had plump seeds, and which also produced high DBC/P values during two subsequent cool, dry seasons. Some selections also had kernels with a thin corneous layer. In the 1974 cool, dry season, all of the selections had low protein values (6-9%). However, in the 1975 cool, dry season, most of the high DBC/P selections had high protein values (12-16%). From a comparison of DBC/P values in lines over seasons, it was apparent that the highest DBC/P progenies were usually coming from high DBC/P parents, indicating that high DBC/P values were heritable. However, the frequency of high DBC/P heads was disappointingly low. Also, many of the high DBC/P selections with low protein subsequently produced progeny with higher protein levels, but with normal DBC/P values.

Crosses between the second high lysine mutant, P-721 and selections from the Ethiopian *hl*-derived lines, were first made at ICRISAT in the 1975 cool, dry season. These crosses produced a much higher proportion of progenies with high DBC/P values than did intercrosses among the Ethiopian *hl*-derived lines. Selection and intercrossing has continued, with emphasis on the P-721 high lysine source, in an attempt to obtain

high yielding lines with high and stable lysine contents.

The following investigations were prompted by questions which were raised while handling the high lysine breeding material. Their basic objectives can be stated as follows:

1) To determine if the DBC/P ratio is sufficiently accurate to estimate lysine in different genotypes and in different environments in the semi-arid tropics.

2) To establish whether the *hl* gene is actually present in a number of lines which were derived from the Ethiopian high lysine parents; to establish whether intercrossing and selection in these lines could increase the lysine content in the progeny; and to evaluate the effectiveness of the P-721 high lysine source in producing agriculturally useful high lysine lines.

3) To investigate the importance of some environmental factors encountered in the semi-arid tropics, in influencing protein, lysine, yield and yield components, and seed vitreousness in a set of lines predominantly normal in lysine.

4) To investigate the response to environments in the Ethiopian *hl*, and P-721 high lysine mutants and in a number of lines derived from crosses involving these parents, and to determine the implications of variations in certain endosperm protein solubility fractions in explaining the responses to environment.

LITERATURE REVIEW

The Need for Cereals with Improved Protein Quality

Examinations of the role of protein quantity and quality in human nutrition are not unanimous in their conclusions. When calculated protein needs were balanced against world protein supplies, a "protein gap" was found, as outlined in Altchul (1972). Calculations were made of the protein needed to meet the physiological need of 97.5 percent of the population, adjusted to take into account the quality of the protein in a national diet. This figure was compared with world protein supplies based on FAO data. Almost all the nine million tonnes of protein deficit, calculated on 1963-65 figures, was in the less-developed countries. Projection to 1985 indicated that 80 million tonnes of additional protein would be required. This increase alone was almost double the 1972 protein supply.

Sukhatme (1972), using data from dietary surveys, came to a similar conclusion:

> "Despite the fact that the average per caput consumption in developing countries as a whole is nearly 40% in excess of the recommended needs . . . so large are the inequalities in protein consumption among the different segments of the population that nearly one third of the people are believed to have a protein intake less than what is needed for maintenance and growth. With the current rate of population growth, the gap between nutrient requirement and actual consumption of protein by the greater part of the population is likely to widen rapidly."

However, studies in which recommended daily allowances were

calculated for the size, age and work load of the population as a whole, and nutrient intakes were calculated from dietary surveys, produced rather different results. Ryan (1977) found that even the lowest income rural groups in all semi-arid tropical states in India had adequate protein and lysine but marginal to deficient levels of calories. Kaul *et al.* (1977) also found that Indian diets were much more limiting in calories than in proteins; all states except the Punjab had a deficit in calorie consumption while the average diet in all states except Tamil Nadu appeared to have adequate protein. Clark (1972) found that 25 to 30 percent of the India population consumed less than adequate calories, while only 2 to 11 percent were below the adequate level for protein. Ryan *et al.* (1975) also examined nutritional data from parts of India, Africa, Thailand and South America, and found that diets in these countries were primarily deficient in calories rather than in protein.

Similarly, Sukhatme (1972) reported from a study of low income households in Tamil Nadu that 32 percent of the diets were deficient in calories, 28 percent were deficient in protein, and that 23 percent were deficient in both calories and protein; however, most of the protein deficient households were also deficient in calories. Since protein utilization is optimum when calories are adequate (Hegsted, 1978), such studies indicate that increasing the intake of existing diets to meet calorie requirements should also provide adequate protein.

The adoption of new varieties can cause a shift in the availability of nutrients. Byers (1976) examined the effects of the green revolution, a term to describe the rapid increase in area and production of cereals in the Indian subcontinent, following the introduction of improved

varieties in the 1960's. He found that the consumption of food grain in India had increased since 1950, but that there had been a decline in per capita availability of pulses, from 70 g per day in 1956 to 36 g per day in 1975, which would indicate that the protein quality of Indian diets had declined. Bressani and Elias (1979) have found that diets in Gautemala have maintained a 10/90 beans/maize ratio for the last twenty years, which they felt was providing marginally adequate protein quality. Any increase in consumption of maize, which could be expected with the use of improved varieties, would result in a lower beans/maize ratio, and possibly in a greater incidence of malnutrition.

However, Ryan and Ashokan (1977), and Namboodini and Choksi (1977) have shown that an increase in productivity of cereals due to the green revolution in India has actually increased the overall availability of both calories and protein, as well as of lysine, even though the production of pulses has declined. Ryan and Ashokan (1977) concluded that increased yield through plant breeding and agronomic improvements should lead to a better balance of nutrient availability. They warned, however, that increases in pulse yields were necessary, "to prevent their rise in price beyond the means of the nutritionally vulnerable and economically poor."

These studies, however, do not specifically assess the status of young children during, or immediately following weaning. This group has the highest relative requirements for calories and protein (Gopalan *et al.*, 1977) and is most prone to diseases associated with nutritional deficiencies. A nutritional survey, carried out in South India with pre-school children, found 50 percent to be deficient in calories

and only 15 percent of the children surveyed were deficient in protein. A feeding study in the same area showed that a group of pre-school children, given a daily supplement with high calories and low protein in addition to their regular food, gained in height and weight compared to the non-supplemented control. Children gained similar amounts whether they had been initially deficient in either calories or protein, which indicated that calories, rather than protein, was the nutritional bottleneck with these children (Sukhatme, 1972). Hegsted (1978) has pointed out that mother's milk is not a high-protein food, containing only 5 to 6 percent of the calories as protein, compared to 10 percent in average adult diets. However, mother's milk is a high quality protein source that is well digested. When a child drinks enough milk to meet its high calories requirements, its higher protein needs are met as well. The problem with weaning is the bulk of food that the child must consume, especially with a largely vegetarian diet which is poorly digested. Sukhatme (1972) felt that the primary cause of Kwasiorkor, as well as Marasmus, may be low food intakes, as special foods are often not prepared for weaning children.

A number of other studies have shown that the quality of the protein is a limiting factor in the diets of children. Bressani (1966) has shown nutritional improvement with pre-school children fed a diet of opaque-2 maize, and Graham (1976) has demonstrated in studies with convalescent malnourished infants that both protein quality and digestibility played important roles in improving the nutritional status of these children. Singh and Jain (1977) examined the effects of supplementing the diets of 18 to 30 month-old children whose home diets were

deficient in calories by about 400 Kcal, but whose protein intake met the Required Daily Allowance. The supplements provided the 400 Kcal deficit, with the protein coming from either skim milk, normal maize or opaque-2 maize. The children fed Opaque-2 maize or skim milk gained more and had greater weight to height ratios than did those children fed either normal maize, or the control group with no supplement. It appeared that the protein quality had an effect on these children even when their total protein intake was adequate.

A review of the lysine fortification of cereals is useful to indicate the effects that might be expected if cereals with genetically improved lysine were to replace the usual source of cereals. A comprehensive review by Jensen (1974) can be summarized as follows. In most studies, animals showed marked nutritional improvement when cereal diets were fortified with amino acids. However, growing animals do have higher growth rates and higher essential amino acid requirements than do children, so such results must be interpreted with some caution. Children who consumed fortified cereal diets showed nutritional improvement (as measured by weight gains, by an increase in nitrogen retention, or by higher serum albumen levels) when they were recovering from malnutrition, or when the percentage of protein calories in their diets had been low (6.4 to 7.3%), or when the cereal diet was the main source of protein.

A large field study has been recently completed on the effects of lysine and iron fortification of wheat flour on 3,000 pre-school children in Central Tunisia where malnutrition was prevalent and where wheat flour provided most of the protein and calories (Boutaline *et al.*, 1977). After four years of fortification, no improvement was found in the

nutritional status of the group fed fortified wheat compared with the control group. Possible reasons given for this lack of response were that illnesses, low calorie intakes, or a low level of zinc may have been overriding problems, or that in fact, high protein quality may not be as important as was previously believed in children.

Although it is becoming clear that several factors are involved in causing malnutrition, there is a clear need for more precise data. In the rural areas of the Semi-Arid Tropics, most food is consumed on the farm where it is produced. Farm-to-farm variation of nutrient content in foods may affect the nutritional status of individuals in rural families. An example can be made using data available for sorghum. Von Oppen (1979) has collected weekly market samples of farmers' grain lots, for three seasons from the wholesale marked in Hyderabad, India. These samples varied in protein content from 5 to 11 percent with a mean of 8.2 percent. Protein content of sorghum samples from 185 households in six villages gave a similar mean and range, with most of the variation occurring among households rather than among villages. Nutritional surveys in India are usually based on the nutrient values of foods provided by Gopalan *et al.* (1977). These authors list sorghum protein at 10.4 percent and lysine as 2.5 percent of the protein. However, the lysine level in normal samples from the sorghum germplasm grown at ICRISAT averaged only 2.1 percent. This apparent overestimation of protein as well as of lysine would give inflated estimates of lysine and protein intakes in village families where sorghum is the main source of protein. Although a preliminary survey of clinical deficiency symptoms of such villages indicated that calories,

vitamins A, C and B complex, calcium and iron were most frequently limiting (ICRISAT, 1977), a closer examination of the nutritional status of individuals in these villages, based on analyses of the nutrients of their actual diets, is now being carried out (Bidinger, 1979).

The High Lysine Genes

Mertz *et al.* (1964) and Nelson *et al.* (1965) first reported that the opaque-2 and floury-2 mutant genes increased the lysine in maize protein by 50 to 70 percent. The demonstration of the superior nutritional value of the protein of these mutants both for laboratory animals (Mertz *et al.*, 1965) and for children (Bressani, 1966) was followed by the incorporation of these mutants into breeding material around the world (Kozubenko, 1968; Wiggin, 1970).

However, a number of problems have slowed the transfer of these genes into commercially acceptable varieties. Reduced kernel weight together with reduced yields were found in opaque-2 lines compared with normal counterparts (Lambert *et al.*, 1969; Sreeramulu and Bauman, 1970; Sperling, 1972), as well as increased susceptibility to ear moulds (Ullstrup, 1971) and lack of acceptability due to floury or soft endosperm (Vasal, 1972). Through persistent breeding efforts, yields of opaque-2 lines up to 86 to 92 percent of their normal counterparts have been reported (Axtell, 1979). Incorporation of vitreous modifiers has increased kernel hardness (Vasal, 1972) and reduced susceptibility to kernel rots (Warren, 1978). Nutritional quality of the modified endosperm lines has been generally reported to be slightly lower than in soft endosperm opaque-2 lines (Gomez, 1975; Gupta *et al.*, 1977).

Similar genes have been discovered that improve the quality of

barley protein. Munck *et al.* (1971) reported screening the barley germplasm collection, using the Udy dye binding technique, and finding the mutant Hiproly, which produced a 30 percent improvement in lysine concentration in the protein, as well as higher protein. However, the endosperm of this high lysine mutant had an undesirable flintiness due to a linked starch binding gene, and the seed weight was one third of the normal barley counterpart (Helm *et al.*, 1974). Ingversen *et al.* (1973) reported several chemically induced high lysine barley mutants. The most promising was named Riso-1508, and was reported to have up to 40 percent increase in lysine with less severe reduction in seed size compared with Hiproly and only a 17 percent reduction in yield compared with its normal parent. Several similar "notch" mutants have been reported by Balaravi *et al.* (1976). Even though Olsen (1978) found a genetically pleiotropic effect of high lysine level and poor seed filling in the Riso mutants, it has been possible to obtain high lysine derivatives with yields almost equal to those of normal barley from backcrosses involving Hiproly (Doll *et al.*, 1974).

In sorghum, the *hl* gene was reported by Singh and Axtell (1973) in two germplasm accessions from Ethiopia. The high lysine *hl* lines known as IS.11758 and IS.11167 contained 3.3 and 3.1 g of lysine per 100 g of protein, an increase of 60-70 percent over normal sorghums. These lines were also reported to contain high protein levels of 17.2 and 15.7 percent. The biological value of the protein of these high lysine sorghums was found to be three times greater than that of normal sorghum, and equal to that of opaque-2 maize. However, the seeds of the high lysine lines were dented, floury and lighter than in normal sorghums.

A second high lysine mutant, called P-721, was identified in sorghum by Mohan (1975), through chemical mutagenesis from a normal line. This mutant appeared to possess a single, semi-dominant gene, which was responsible for an increase in lysine (as percent of protein) of 60 percent. The biological value of the protein of this mutant was shown to be almost equal to that of IS.11758 and IS.11167. As well as increasing lysine, the balance of leucine to isoleucine appeared to be improved in both *hl* and P-721 mutants (Singh and Axtell, 1973; Mohan, 1975), and nicotinic acid content of the *hl* mutants was shown to be higher than normal (Pant, 1975). These attributes may be desirable in reducing the incidence of pellagra among sorghum-eating people (Belavady, 1975).

However, the problems in the high lysine sorghum mutants are similar to those of the high lysine maize and barley mutants. In spite of large head size and high seed number per head, the yields of Ethiopian high lysine farmers' lines were about 25 percent lower than similar farmers' lines with normal lysine (Axtell, 1979). The kernel weight of P-721 was found to be about 25 percent lighter than that of its normal parent (Mohan, 1975). Axtell *et al.* (1979) reported that the grain filling period in P-721 was reduced, producing lower kernel weights and consequently approximately 10 percent lower yields. The floury endosperms of the high lysine sorghums have been found to give lower milling yields and a less acceptable product (Anderson *et al.*, 1977; Murty, 1979). Considerable breeding work has attempted to correct these deficiencies. Axtell *et al.* (1979) reported data suggesting that selection for yield in appropriate crosses with P-721 would result in compensation for low kernel weight by increased number of kernels per head,

and number of heads per hectare, to produce higher yielding, high lysine derivatives. High lysine P-721 derivatives with modified vitreous endosperms have been reported by Ejeta (1979) using parents which possessed genetic factors for modification. These modified endosperm derivatives with high lysine had heavier heads than the P-721 parent, and appeared to have higher yields, but slightly lower lysine and protein percentages. Singh (1976) reported vitreous endosperm plump kernels with high lysine in F_2 and F_3 heads from crosses with the Ethiopian *hl* mutants. However, the protein levels in these segregates were about half that of the *hl* parent, making it questionable whether the high lysine gene or simply the low protein content was responsible for the high lysine expression.

In most of the high lysine mutants found in various cereal species, the change in amino acid composition occurs primarily or entirely in the endosperm rather than in the embryo. This has been found to be the case with opaque-2 and floury-2 maize (Nelson, 1969), Riso 1508 barley (Tallberg, 1977), and *hl* and P-721 sorghum mutants (Singh and Axtell, 1973; Mohan, 1975). A striking feature of the endosperm of these mutants is the change in the proportion of the different solubility fractions which make up their proteins. The alcohol soluble or prolamine fraction which contains almost no lysine, has been found to be greatly reduced. This may be the primary effect of these mutants (Nelson, 1979). The reduction in the prolamine fraction was accompanied by increased amounts of the water and salt soluble fractions called albumins and globulins, which are rich in lysine. In some cases an increase occurred in the alkaline soluble glutelin fraction which has an intermediate lysine level (Nelson, 1979).

Examining the protein fractions in sorghum endosperm, Guiragossian *et al.* (1978) found that prolamine accounted for 50 percent of the protein in normal sorghum and for only 25 percent in P-721 and 30 percent in IS.11167. Consequently, a three-fold increase in nitrogen occurred in the water and salt soluble fractions, and a small increase in the glutelin fraction. Comparable results had been found earlier by Jambunathan *et al.* (1975) using whole seeds. It appeared unlikely that new types of protein were formed in the mutants, nor did it appear that the amino acid composition within the fractions was altered (Guiragossian *et al.*, 1978; Paulis and Wall, 1978; Tallberg, 1977; Nelson, 1979; and Sodek and Wilson, 1971). The *lys* gene from Hiproly barley appeared to be rather different in that it resulted in only a slight reduction of the prolamine fraction, but in greater production of four water soluble polypeptides (Munck, 1972). In the maize and barley mutants, non-protein nitrogen and free amino acids were also found to increase (Gupta *et al.*, 1977; Brandt, 1976). In IS.11758 high lysine sorghum, however, free amino nitrogen was reported to be equal to that of a normal Indian hybrid (Mehta *et al.*, 1979).

The high lysine mutants in maize and sorghum also have an altered endosperm structure. In maize, endosperm structure is created by a protein matrix in which starch granules and protein bodies are imbedded. Prolamines are primarily located in cytoplasmic organelles as protein bodies while the matrix is mainly glutelin (Seckinger and Wolf, 1973). Wolf *et al.* (1967) have shown that the size of the protein bodies in the endosperm of Opaque-2 maize was greatly reduced. This has been shown to be the case in sorghum for the Ethiopian *hl* and P-721 mutants by Rooney

and Sullins (1977), who also found that the high lysine sorghums contained relatively little protein matrix except around the periphery of the endosperm. Baezinger and Glover (1977) also found the protein matrix to be thinner in Opaque-2 maize and suggested that the protein matrix may be involved in the expression of kernel density and vitreousness. A matrix that bound the starch granules tightly, resulting in fewer air spaces, was found to produce a vitreous kernel (Robutti *et al.*, 1974).

Environmental factors are also important in determining the degree of vitreousness. Tsai *et al.* (1978) found increased vitreousness as well as increased prolamine in maize kernels as fertilizer nitrogen was increased. Navarro (1976) found that the location affected the degree of modification in Opaque-2 modified lines, with cooler temperatures producing a greater degree of modification. In sorghum, Ejeta (1979) was able to find modified P-721 derivatives with high lysine, which appeared to have a stable expression across generations and seasons. He also reported that phenotypic expression of vitreousness varied with location, as in some cases vitreous high lysine selections made in Puerto Rico produced opaque seeds when planted in Indiana, U.S.A.

In addition to the qualitative high lysine genes which have been discussed above, other sources of high lysine have been reported. Choe *et al.* (1976) were able to increase the lysine content in maize through a program of recurrent selection. The expression of high lysine in these derived lines appeared to be independent of the Opaque-2 gene. Protein in the high lysine lines was also higher. Virupaksha and Sastry (1968) reported finding a high lysine, high protein sorghum line from the world collection. Although the inheritance of lysine in this

line was not reported, it appeared that there was only a slight reduction in prolamine, and an increase in the glutelin fractions in this line.

Correlations between Protein and Lysine, and between Protein and Yield in Lines Grown in One Environment

In 1951, Frey found a negative protein-lysine correlation in maize, and showed that it resulted from an increase in the lysine-poor prolamine or zein protein fraction in the higher protein selections. This negative relationship has also been found in sorghum (Rana and Murthy, 1975; Virupaksha and Sastry, 1968; Deosthale *et al.*, 1970). Ejeta (1976) compared the protein-lysine regression in both high and normal lysine sorghums collected from Wallo, Ethiopia and grown out at one location at Alemaya, Ethiopia. A negative slope was found for both groups, but a steeper negative slope was associated with the high lysine group than with the normal group. A highly significant negative correlation was found for both the normal lysine and high lysine sorghum groups.

In rice (Juliano, 1972), wheat (Laurence *et al.*, 1958), and wheat and barley (Woodham *et al.*, 1972), the negative correlation between protein and lysine was found only at low and medium protein levels; in high protein lines, lysine and protein were uncorrelated. In oats, globulin proteins, whose amino acid composition is similar to that of the total seed protein, are the chief storage proteins. Therefore, increasing the protein content in oats resulted in an increase mainly of the globulin fraction, and consequently the amino acid balance was not affected (Peterson, 1976). In all other cereals, however, the evidence indicates

that selection for higher protein content can be expected to result in a poorer quality protein.

A negative correlation has been found between protein and yield in most cereals. This is not surprising since it has been calculated that the energy required by the plant to produce one unit of protein is more than twice that required to produce one unit of carbohydrate (Sinclair and de Wit, 1975). However, higher yielding oat varieties wich maintained a high protein level have been reported (Frey, 1975) as well as a higher protein wheat, Atlas 66, which maintained a high grain yield (Middleton *et al.*, 1954). This fortunate combination of high yield and high protein in the Atlas line was found to be due to a more rapid accumulation of nitrogen in the head following flowering (Seth *et al.*, 1960); a better translocation ability from the plant to the developing seed (Johnston *et al.*, 1967); and an extended period of protein accumulation which continued as the seed was drying (Brunori *et al.*, 1977).

Environmental Effects on Protein and Lysine Levels

Protein and lysine levels in cereals can be greatly affected by the environment in which they grow. Site to site differences in protein were found to be larger than varietal or hybrid differences in wheat grown in Nebraska (Terman *et al.*, 1969), maize in Virginia (Genter *et al.*, 1956) and sorghum in Kansas and in India (Miller *et al.*, 1964; Deosthale and Mohan, 1970). In each case, the varieties or hybrids were local or adapted genotypes being tested for potential release. However, Campbell and Pickett (1968) were able to show a larger variance due to genotype than due to environment, when the genotypes were deliberately chosen to have a wide range of protein, and were tested over only a few environments.

Soil Nitrogen. Application of nitrogen fertilizer to the soil can have a great effect on protein as well as on yield of cereal crops. Johnston *et al.* (1973) showed that increments of nitrogen fertilizer caused a linear increase in protein in both a high protein and normal protein line even up to the highest level of nitrogen (135 kg/ha), with the high protein line maintaining two percent higher protein at all levels of applied nitrogen. Increments of nitrogen also caused a yield increase in both lines. The maximum yield, however, occurred at 90 kg of nitrogen for the normal line and 112 kg/ha for the high protein line. Concurrent increases in protein as well as in yield with increases of nitrogen fertilizer have usually been the case in sorghum (Miller *et al.*, 1964; Burleson *et al.*, 1956) and in corn (Gomez, 1975; MacGregor *et al.*, 1961). In spring wheat in western Canada, low or moderate rates of applied nitrogen have been found to reduce protein levels (Partridge and Shaykewich, 1972; Dubetz, 1972). Partridge and Shaykewich (1972) reasoned that the protein was being diluted by the large increases in yield due to the nitrogen fertilizer. With sorghum in India, Deosthale *et al.* (1972) reported that nitrogen fertilizer increased yield at all rates, but only increased protein at rates greater than 135 kg/ha. Terman *et al.* (1969) examined the response of wheat nurseries in Nebraska over several years, and found that the first effect of nitrogen fertilizer was to increase yield if water and other factors were not limiting; when soil nitrogen levels were high, additional nitrogen increased the protein percent. Similarly, Warsi and Wright (1973) found that nitrogen applied at planting to sorghum in India was mainly effective in increasing yield, while a later application was mainly effective in increasing protein content.

Heavy applications of nitrogen have been found to reduce seed size in wheat (Gardner and Jackson, 1976), and in sorghum (Roy and Wright, 1973). In these cases the yield increases which were found were due to increased numbers of both tillers per hectare and seeds per head in wheat, and increased numbers of seeds per head in sorghum.

Lysine, expressed as a percent of protein, decreased in maize, barley, and sorghum with increased nitrogen application (Rendig and Broadbent, 1979; Andersen and Koie, 1975; Deosthale, 1972). This was associated with a concurrent increase in protein, and is part of the negative protein-lysine relationship that has been previously pointed out to exist in all cereals except oats. However, lysine content, expressed as percent of the sample, has been found to increase with applications of nitrogen fertilizer (Gunthardt and McGinnis, 1957; Waggle *et al.*, 1967; Sauberlich *et al.*, 1953). MacGregor *et al.* (1961) however, found that the increase in protein was balanced out by the decrease in lysine (as percent of protein) and that nitrogen fertilizer did not affect the lysine content of maize.

Schneider *et al.* (1952) found that maize, when grown under a high rate of nitrogen fertilizer, produced kernels with a higher embryo/endosperm ratio compared with kernels grown under low nitrogen conditions. The protein solubility fractions for both embryo and endosperm were then determined. The fertilizer nitrogen treatment had little effect on protein fractions in the embryo, as about half the embryo protein was made up of high quality albumens and globulins in both nitrogen treatments. In the endosperm, the high nitrogen treatment resulted in an increase of the lysine-poor prolamine fraction, and a decrease in the

glutelins, albumens and globulins. Similar results were obtained in sorghum by Warsi and Wright (1973) who found that a high rate of nitrogen fertilizer increased the prolamine fraction and decreased the lysine in the protein.

The response to fertilizer nitrogen has been found to differ in the high lysine mutants of both maize and barley compared to normal types. Andersen and Koie (1975) found that with high levels of nitrogen, the protein content of Riso high lysine barley increased less than in the normal Bomi parent. Similarly, in maize, Zink (1979) found that nitrogen applied after anthesis produced a smaller increase in the protein of an opaque-2 hybrid compared to a normal maize hybrid.

Lysine levels (as percent of protein) in both Opaque-2 maize and Hiproly barley were found to be unaffected by increased nitrogen levels, while lysine in the seeds of normal lines of both cereals declined (Sonntag and Michael, 1973). More recent experiments have confirmed that with increased levels of nitrogen, lysine levels in the high lysine mutants of maize and barley were lowered to a lesser extent (Zink, 1979; Andersen and Koie, 1975) or not altered (Zink and Wilberg, 1976) compared with a decline in lysine in normal lines. In both maize and barley high lysine mutants, the prolamine content has been found to remain almost constant with increasing nitrogen levels (Zink and Wilberg, 1976; Andersen and Koie, 1975). It was the opinion of most of these authors that the relative constancy of the lysine levels in the high lysine mutants of maize and barley should lead to enhancement of the improved nutritional quality of the mutants compared to normal lines when grown with higher levels of nitrogen.

Other Fertilizer Elements. Phosphorus has been found to have no effect on protein content in maize (Genter *et al.*, 1956) or in sorghum (Roy and Wright, 1973) even though yield was increased, and more nitrogen per unit area was taken up. Neither phosphorus nor potassium application was found to affect amino acid composition or protein of cereals (Eppendorfer, 1975). Potassium has been shown to improve the translocation efficiency of plant nitrogen into the grain, as well as the uptake of soil nitrogen into the grain at later stages of grain filling, but no particular protein fraction was preferentially supplied; consequently no change in nutritional quality occurred (Koch and Mengel, 1977).

Moisture Stress. Moisture stress has been found to increase protein (Dubetz and Bole, 1973; Campbell, *et al.*, 1977), and to decrease both the number of tillers as well as the grain size in spring wheat. Under dry conditions, nitrogen applications were not effective in increasing protein in wheat (Hutchinson and Paul, 1966) or in maize (Genter *et al.*, 1956). In Norway however, an increase in protein, as well as a decrease in lysine and in the albumen and globulin fractions of wheat protein, was found with a dry soil-high nitrogen treatment (Kolderup, 1975).

The effect of rainfall on protein content of maize was examined by Earle (1977) in commercial lots of maize grown in the United States from 1907 to 1972. He found a negative relationship between protein content and July rainfall, a less negative relationship between protein content and August rainfall, and no relationship with September rainfall. In India, supplemented irrigation was found to reduce the protein percent of wheat, but because yields were increased, the total uptake of soil nutrients also increased (Hazra *et al.*, 1975; Varma, 1976). Tsoi

(1975) found that the protein content of maize in Russia was reduced from 11.5 percent to 8.5 percent with irrigation. However, irrigation of sorghum appeared to reduce the variation in protein content compared to rainfed conditions, but not the mean protein. Rainfall was reported to have no effect on protein content in sorghum when grown at 24 research stations in India (National Institute of Nutrition, 1973).

Air Temperature. Increases in air temperature up to 24°C were found to reduce the kernel weight and yield of wheat, to increase the protein content, and to decrease the percent lysine in protein (Kolderup, 1975). Earle (1977) found that there was a positive relationship between the protein content in maize grown in the United States and mean July temperatures above 24°C. Similarly, Worker and Ruckman (1968) found that protein content of sorghum grain grown in the south-western desert area of California from 1961 to 1966 increased as the air temperature for the 20 days after anthesis increased. Sorghum planted in April with cool temperatures and maturing under hot temperatures had a relatively low protein content (10.1%), while July plantings in hot temperatures, maturing in cooler temperatures, had a high protein content (14.5%). These authors also found a negative protein-yield relationship over seasons. This contrasts with the positive protein-yield correlations found with changes in fertilizer nitrogen, previously reviewed.

Soil. MacGregor *et al.* (1961) examined the effects of two soil types on protein and lysine content in maize. They found higher protein and lower lysine (as percent protein) in a heavier textured and more fertile loam soil compared with a less fertile, loamy, fine sand in Minnesota.

Genotype x Environment Interactions and Stability

The existence of differences in rankings in performance of varieties in different environments has led to a large number of studies of the magnitude and nature of the interaction. Reviews have been made by Comstock and Moll (1963), Allard and Bradshaw (1964), and Moll and Stuber (1974). These have attempted to "help the plant breeder in developing varieties which minimize unfavourable genotype-environment interactions, i.e., varieties which are able to control their developmental process in such a way as to give high and consistent performance" (Allard and Bradshaw, 1964). These authors concluded that a variety can achieve stability either by population buffering--where several genotypes comprise the variety, each adapted to a specific set of environments or a homozygous line can possess individual buffering which is a form of homeostasis, (Comstock and Moll, 1963) in which individual plants perform more consistently in different environments.

Comstock and Moll (1963) pointed out that the genotypic variance when estimated at a single site, is biased upwards by the inclusion of the genotype by environment (G x E) component, and therefore the G x E component would be expected to increase as the reference base of locations is expanded (Abou el-Fittouh *et al.*, 1969). Schutz and Bernard (1967) found that the genotype by year interaction was smaller than genotype by location interaction for yields of soybeans, and that locations could be substituted for testing over years.

For grain yield in sorghum, Laing and Walter (1966) found that genotype by year and genotype by location interactions were more important than either the year or location main effects. However, as regards

protein, several studies have shown that the G x E interaction is quite small or non-significant in maize (Genter *et al.*, 1956; Miller *et al.*, 1964), soybeans (Schutz and Barnard, 1967), wheat (Johnson *et al.*, 1973; Terman, 1979), wheat and barley (Hadjichristodoulu and Della, 1978), as well as in sorghum (Campbell and Pickett, 1968; Shaffert *et al.*, 1972). In some cases, however, usually where large sets of both environments and genotypes were tested, substantial genotype by environment interactions for protein were found. This was true in wheat (Stroike and Johnson, 1972), barley (Pomerantz *et al.*, 1977), and sorghum (Deosthale *et al.*, 1972; Deyoe and Shellenberger, 1965).

The different response in lysine levels, producing a G x E interaction in normal lysine and high lysine genotypes of maize and barley which were grown at different nitrogen levels has already been discussed. Stroike and Johnson (1972) found G x E interaction present in winter wheat lines, with low lysine lines being more responsive than higher lysine lines. In sorghum, Campbell and Pickett (1968) found that the G x E for lysine was important, since late maturing lines tended to be shrivelled and produced higher lysine. In contrast, Shaffert *et al.* (1972) found a low level of G x E interaction for lysine while Deyoe and Shellenberger (1965) found no G x E interaction for several amino acids, including lysine.

Attempts have been made to utilize the G x E variance component to gain information on the stability of individual lines over environments. Baker (1969) divided the G x E component into single degree of freedom comparisons for each spring wheat line under test, and Plaisted and Peterson (1959) calculated a separate G x E component for all possible pairs of lines being tested. However, these methods do not

distinguish among lines on the form of response across environments. Byth *et al.* (1977) used cluster analysis to provide patterns of genotype and environment response in large data sets, to examine the dynamic nature of genotype response. The lines under test with similar responses were grouped together so that those lines which produced the most desirable response over environments could be chosen. The answer to the question of the criteria to be used in defining a desirable genotype in a set of environments is left to the individual researcher.

Stability analyses of individual genotype responses most often utilize regression techniques. Several methods of regression of individual genotype means on an environmental index to provide stability parameters were reviewed by Freeman (1973). Yates and Cochran (1938) first proposed the regression of the location mean of each of the entries onto the means of all the varieties at a location. Findlay and Wilkinson (1963) and Perkins and Jinks (1968) have more recently used similar models, as did Eberhart and Russell (1966). Eberhart and Russell (1966) described the stability of a genotype in terms of three stability parameters: mean yield over environments, the regression coefficient (β) which measured response over environments, and the sums of deviations squared (ΣS^2) which measured the failure of linear regression to account for the variety response. A stable variety was defined as having a high mean yield, a regression coefficient (β) of one, and deviations from regression as small as possible. Kaltsikes and Larter (1970) examined the stability of durum wheat lines in Western Canada, using the methods of Eberhart and Russell (1966),

Perkins and Jinks (1968) and Findlay and Wilkinson (1963) and found that all three methods gave satisfactory estimates of stability parameters.

A number of criticisms of the regression approach have been made. Knight (1970) pointed out that even though the linear regression component does account for a major portion of the G x E variance, a linear relationship may poorly describe the performance of lines at super- or sub-optimal conditions. Several authors have pointed out that using the means of the genotypes as a measure of the environmental index, makes the response of the genotypes dependent on the particular set of genotypes used. Wood (1967) attempted to use independent soil and plant variables as a measure of the environment, but was only able to account for a limited portion of the environmental variation. Tan *et al.* (1979) used the mean of the parent genotypes of smooth brome grass to form an independent index on which the progenies of these parents were regressed. They found that the ranking of the regression coefficients of the progenies did not change whether or not they used an independent environmental index, which indicated that an index formed from a large number of genotypes under test should be satisfactory.

A considerable number of investigations have been carried out using Eberhart and Russell's analysis. Bains (1976) found that the stability (as measured by the regression coefficient) of progenies of spring wheat crosses corresponded very closely to their parents. Segregation for linear response or β value only occurred in progeny of crosses where parents had different β values, which indicated that this stability parameter was under genetic control. Similarly, Frey (1972)

used stability analysis to show that isolines of oats having different crown rust genes had different environmental responses. Reich and Atkins (1970) found that the yield of hybrid blends were more stable than parents of single crosses of grain sorghum. Jowett (1972) and Patanothai and Atkins (1974) found that yields of three-way hybrids were generally more stable than single cross hybrids but that high yielding, stable single cross hybrids could be identified. Stroike and Johnson (1972) examined the stability of several traits, including protein and lysine in winter wheat lines grown in an international array of environments. They found that higher protein lines were generally more responsive than were lower protein lines, and that low lysine lines were more responsive than higher lysine lines.

Stable genotypes whose stability is due to individual buffering or homeostasis should, according to Allard and Bradshaw (1964), have less plant-to-plant variation compared with less stable genotypes. However, Francis and Kannenberg (1978[b]) found that standard deviations of ear weights of maize hybrids, calculated from 10 ears per plot, had no relationship with their stability parameters. Arnold *et al.* (1977) found that standard deviations calculated on 62 ears per entry was three times higher for lysine in the opaque-2 maize population compared with a normal population. In terms of plant-to-plant variability, the expression of lysine in the opaque-2 population was less stable. Coefficients of variation (CV's) calculated on genotype values across environments have also been examined as a possible measure of stability. Francis and Kannenberg (1978[a]) found that corn hybrids with low CV's and high yields, also had low deviations from regression and a regression co-

efficient close to one. However, Binswanger and Barah (1979) using theoretical data, found no relationship in the ranking of CV's with other stability parameters.

The occurrence of a marked change in a trait over generations has also been termed instability, but is often due to a genetic change occurring in the progeny. Pollacsek (1970) found a dominant suppressor gene in crosses between normal and Opaque-2 maize lines which gave vitreous, normal-lysine progeny. Ejeta (1979) found that seeds of high lysine Opaque P-721, which had undergone an additional mutagenic treatment, produced a high proportion of normal, vitreous types. He proposed that this was due to the introduction of an additional suppressor mutation rather than a reversion of the original high lysine mutation.

In examining the effects of CV's associated with error terms in field experiments, Ulonska and Baumer (1976) found a reduction in CV for yield and protein at higher levels of fertilizer nitrogen and concluded that successful selection for both yield and protein is only possible under saturated nitrogen conditions. This would assume that there is no genotype x nitrogen interaction in the material tested. Hadjichristodoulu and Della (1978) compared variances for crude protein at different levels of nitrogen and irrigation in wheat and barley. In contrast to the above authors, they found that an intermediate rate of nitrogen (89 kg N/ha) produced the lowest variances.

Use of the Udy Dye Binding Technique to Estimate Lysine Concentrations in Protein

The UDY acid orange-12 dye binding technique was first used to estimate rapidly the crude protein in wheat (Udy, 1954) and later in other cereals (Udy, 1971). The dye binds quantitatively with the basic

amino acids arginine, histidine and lysine, as well as with free end α amino groups of the proteins. The precipitated protein is filtered out and the amount of decoloration is measured (Munck, 1972). It was found accidentally that heat-damaged barley in which the lysine was largely destroyed, had lower dye binding capacity (DBC) values than did undamaged barley, and that DBC values correlated well with mouse growth (Munck, 1972).

Mossberg (1969) showed that DBC values were more closely correlated with basic amino acid content than with protein content in several cereals, and that lysine and basic amino contents were closely correlated. He recommended the DBC technique for screening for lysine in conjunction with microkjeldahl determinations to adjust for protein level. This method was used in identifying high lysine barley mutants (Munck, 1972). More recently Laberge *et al.* (1976) regressed the DBC values of high lysine and normal barley parents on their protein values and found a distinctly different regression line for each parent. It was found possible to select high lysine progeny which had small deviations from the regression line of the high lysine parent. These authors also found that lysine values estimated from DBC vs. lysine regression gave a close fit with lysine determination made by the amino acid analyzer (AAA).

Christensen (1977) at Purdue, used a regression of DBC on protein in order to produce adjusted DBC values which were uncorrelated with protein in sorghum. He also found some evidence for non-linearity of the DBC-protein regression.

Meckenstock (1979) calculated DBC-protein regression equations

for a number of families generated from a sorghum-breeding program, and used the intercept value with the x axis as a measure to pick out those families which were likely to produce higher lysine selections. In comparing ways of using the DBC technique in screening for high lysine, he found that selection on the basis of DBC/protein ratio was effective, based on close correlation with AAA lysine values. One family, however, which had been selected for high DBC, produced high histidine values rather than high lysine.

At ICRISAT, Jambunathan (1978) found that the ratio of DBC/ protein percent (DBC/P) gave a close correlation (.93) with AAA lysine determination in sorghum germplasm samples having a wide range of protein and lysine. From the regression of DBC/P values onto AAA lysine, a prediction equation was developed to estimate a lysine value from DBC/P ratio. Although this estimated lysine value held fairly well for both high and low protein samples, Jambunathan (1978) did find that samples with high protein were slightly overestimated, while low protein samples were being slightly underestimated.

MANUSCRIPT 1

THE USE OF THE UDY DYE BINDING CAPACITY TECHNIQUE TO ESTIMATE LYSINE PERCENT IN PROTEIN IN HIGH AND NORMAL LYSINE SORGHUM GENOTYPES GROWN IN DIFFERENT ENVIRONMENTS

INTRODUCTION

The UDY acid orange-12 dye used in the dye binding capacity analysis (DBC), binds quantitatively with the basic amino acids, arginine, histidine and lysine, as well as with the free end amino group of proteins. Mossberg (1969) recommended the use of the DBC value, in conjunction with a micro-kjeldahl protein determination, to screen for higher lysine levels in barley. This method was used by Munck (1972) in identifying high lysine barley mutants. More recently Laberge *et al.* (1976) developed a prediction equation based on the regression of DBC values on actual lysine values from amino acid analysis (AAA), and found a close agreement between lysine contents predicted from DBC values and the AAA lysine values.

Meckenstock (1979) compared ways of using the DBC procedure for screening sorghum for higher lysine content. He found that, in selecting individual heads, the size of the residuals from a DBC-protein regression line was closely related with lysine percent in protein, so that values above the regression line were higher in lysine. Meckenstock also found that selection on the basis of DBC/protein percent (DBC/P) ratio to be a good method of selection.

At ICRISAT, Jambunathan (1978) found that the DBC/P ratio gave a close correlation (.93) with AAA lysine percent in protein, based on 100 sorghum samples with a wide range of protein and lysine. From the regression of AAA lysine values on DBC/P ratios, he developed a prediction equation to estimate lysine percent in protein.

Although this estimated lysine value held up fairly well for both high and low protein groups, he did find that the estimated lysine

values in samples with low protein were slightly underestimated, while those in high protein samples were slightly overestimated.

In the course of a project, based at ICRISAT, to evaluate the environmental response and stability of lysine and protein in a number of high lysine and normal sorghum lines, a further evaluation of this method of estimating lysine was made.

In any experiment, it is important to keep the unexplained, or error variation, as low as possible. Laboratory variation was monitored during the course of this thesis study by examining the variation in hidden checks from a single seed lot. Also, a comparison of the contribution of field variation, variation due to sampling a seed lot, and variation due to laboratory error was carried out.

MATERIALS AND METHODS

A prediction equation was developed, which contained both a DBC/P term and an independent protein term to make the error in estimating lysine independent of the protein percentage in the 100 samples used by Jambunthan (1978). The estimated lysine values derived from this 2-term equation were then compared with AAA lysine values in samples of genetically high and normal lysine sorghums, grown in environments which produced both high and low protein values. In an initial set of 10 samples, estimated lysine values were compared with AAA lysine readings from the Beckman 120C amino acid analyzer at ICRISAT. Subsequently, the estimated lysine values in 56 samples, from environments that produced either high or low protein, were compared with AAA lysine readings from the Beckman 121 amino acid analyzer at the Plant Science Department of the University of Manitoba, as the ICRISAT Beckman machine was not accessible for these analyses. However, this provided an opportunity to compare lysine values from the two Beckman instruments.

In order to monitor laboratory error in estimating protein and DBC values at ICRISAT, hidden checks from a common seed lot were routinely submitted after every 75 samples and the variation in the check sample was calculated.

A comparison of the error variation due to field heterogeneity, sampling, and laboratory determination was made at ICRISAT during the 1978 warm wet season, by taking 3 lines from a relatively uniform site, termed a high management black soil field (HMB), and from a heterogeneous site, termed a low management red soil field (LMR), with 4 replicates at each site. The grain from each plot was thoroughly mixed,

and 2 samples were taken from each plot and submitted to the ICRISAT biochemistry laboratory. Two determinations were run for both protein percent by the Technicon method (N x 6.25) (Mitcheson and Stowell, 1969) and for DBC for each sample submitted. Variances and CV's were calculated from the estimated mean squares and error terms using a nested design analysis of variance.

RESULTS AND DISCUSSION

The regression analysis of AAA lysine *vs* 2 independent variables, viz: DBC/P and protein percent, carried out on 100 sorghum lines grown at ICRISAT, produced the following prediction equation:

$$Y = .1507 + .664\ X_1 - .023X_2$$

where Y = estimated lysine

X_1 = DBC/P

X_2 = protein percent

with a coefficient of determination (r^2) of 0.894. The residuals of AAA lysine - estimated lysine were, then, independent of the protein value of the sample.

A comparison of AAA lysine values obtained through analysis at ICRISAT, with the estimated lysine values produced by the prediction equation, are presented in Table 1.1 from a set of 4 lines grown in two different environments, with or without nitrogen topdressing (HN or LN). Data from the same set, with the addition of another 2 samples, are presented graphically in Figure 1.1.

From these rather limited numbers, it did appear that the estimated lysine values were predicting the actual lysine values, as measured by the ICRISAT 120C Beckman amino acid analyzer, with a fair degree of accuracy ($r = 0.85$). Also, residuals of the AAA lysine - estimated lysine values were both positive and negative, which did not indicate any bias towards over or underestimation of lysine values. However, the residual levels of the two samples with highest lysine values were larger than was desirable.

The comparison of estimated lysine values with lysine values from

TABLE 1.1. A comparison of two methods of lysine analysis carried out at ICRISAT.

Line	Nitrogen[a]	Protein % (N x 6.25)	AAA lysine[b] (% of protein)	Estimated lysine[c] (% of protein)	Difference (residuals)
Q.50890	HN	10.80	1.97	1.97	0
	LN	6.03	2.47	2.59	-.08
CSH-1	HN	13.18	1.71	1.85	-.13
	LN	4.95	3.02	2.71	+.31
Q.50662	HN	12.49	2.48	2.64	-.16
	LN	9.30	2.60	2.76	-.16
P-721	HN	15.81	2.62	2.93	-.31
	LN	9.87	2.44	2.36	+.08

[a] LN samples from plots with 20 kg N/ha at planting. HN plots received an additional 150 Kg N/ha in top-dressed applications.

[b] Lysine values from Beckman 120C Amino Acid Analyzer at ICRISAT.

[c] Lysine values estimated from a linear regression equation using dye binding capacity/protein percent and protein percent as independent variables.

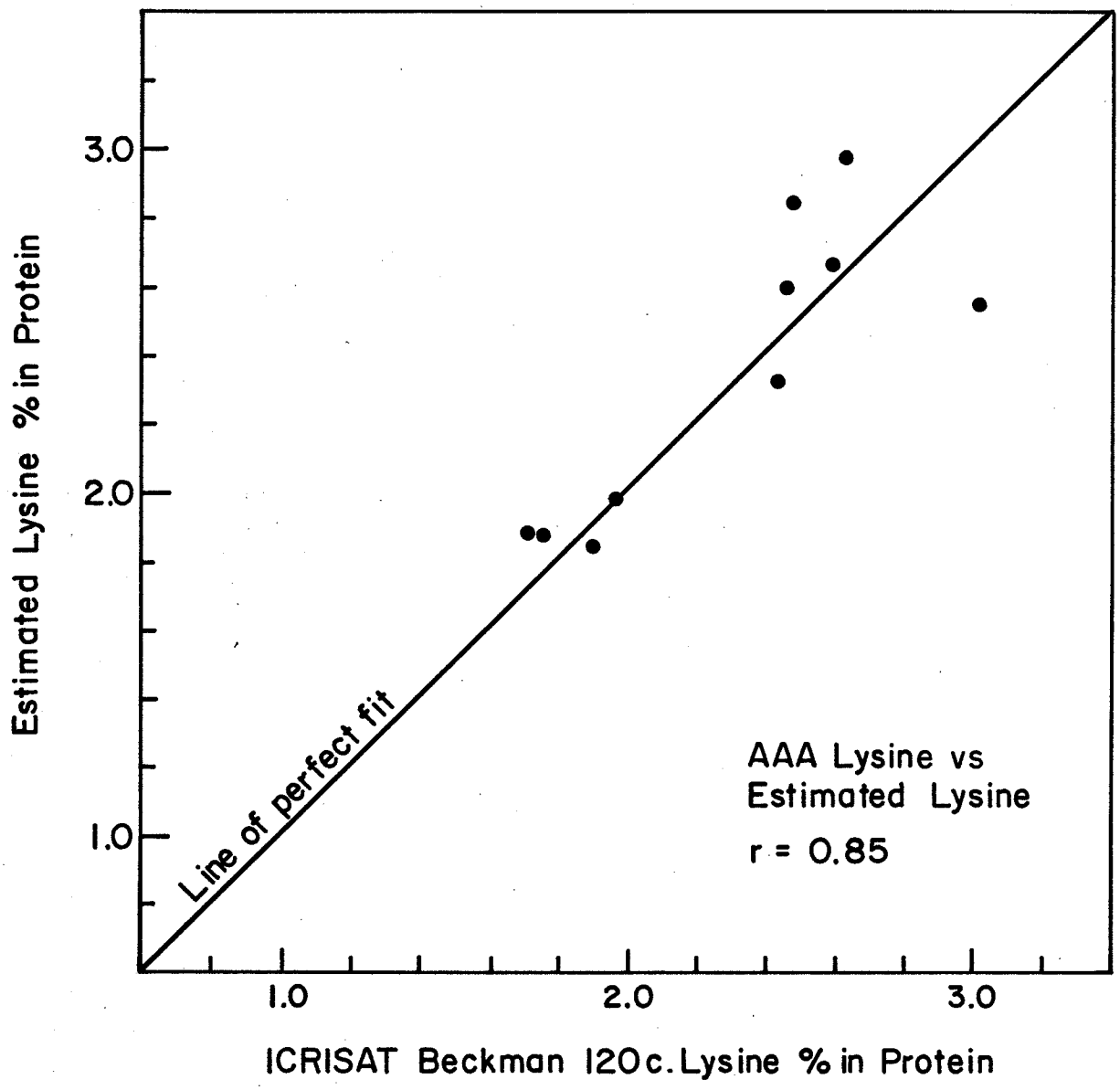

Figure 1.1. Comparison of estimated lysine values with AAA lysine values from the ICRISAT Beckman 120c amino acid analyzer using samples with a wide range of protein and lysine contents. Protein = N x 6.25.

the Beckman 121 amino acid analyzer at the University of Manitoba Plant Science Department is presented in Figure 1.2, using 56 samples from environments which produced both high and low protein values. Most estimated lysine values were lower than the AAA lysine levels obtained, lying below the line of perfect fit. Four samples appeared to be exceptional, producing higher estimated than AAA lysine values. These were samples of two lines Q.50662, and Q.50687, the grain of which was characterized by small bulges opposite the embryo, near the base of the kernel. It was at first thought that the high estimated lysine values of these lines might be due to high levels of basic amino acids other than lysine. However, a re-analysis of seven samples for all basic amino acids (Table 1.2) indicated that both Q.50662 and Q.50687 had moderately high lysine levels, but did not have higher values of either histidine or arginine, than did the other samples. The high estimated lysine values in these two lines might be due to their smaller grain size which would result in a higher proportion of seed coat tissue in these samples. The possible binding of the UDY dye with some of the complex molecules in the seed coat would explain the higher estimated lysine compared with AAA lysine values.

The correlation of estimated lysine vs. AAA lysine calculated on all 56 samples was $r = 0.85$. When the samples of the two bulgy lines were removed the correlation value increased to $r = 0.94$. In this set of 52 samples, the mean of the estimated lysine values was .31 percent lower than the University of Manitoba Plant Science Beckman lysine values. Since there was no evidence that the ICRISAT Beckman lysine values were lower than the estimated lysine values (Figure 1.1),

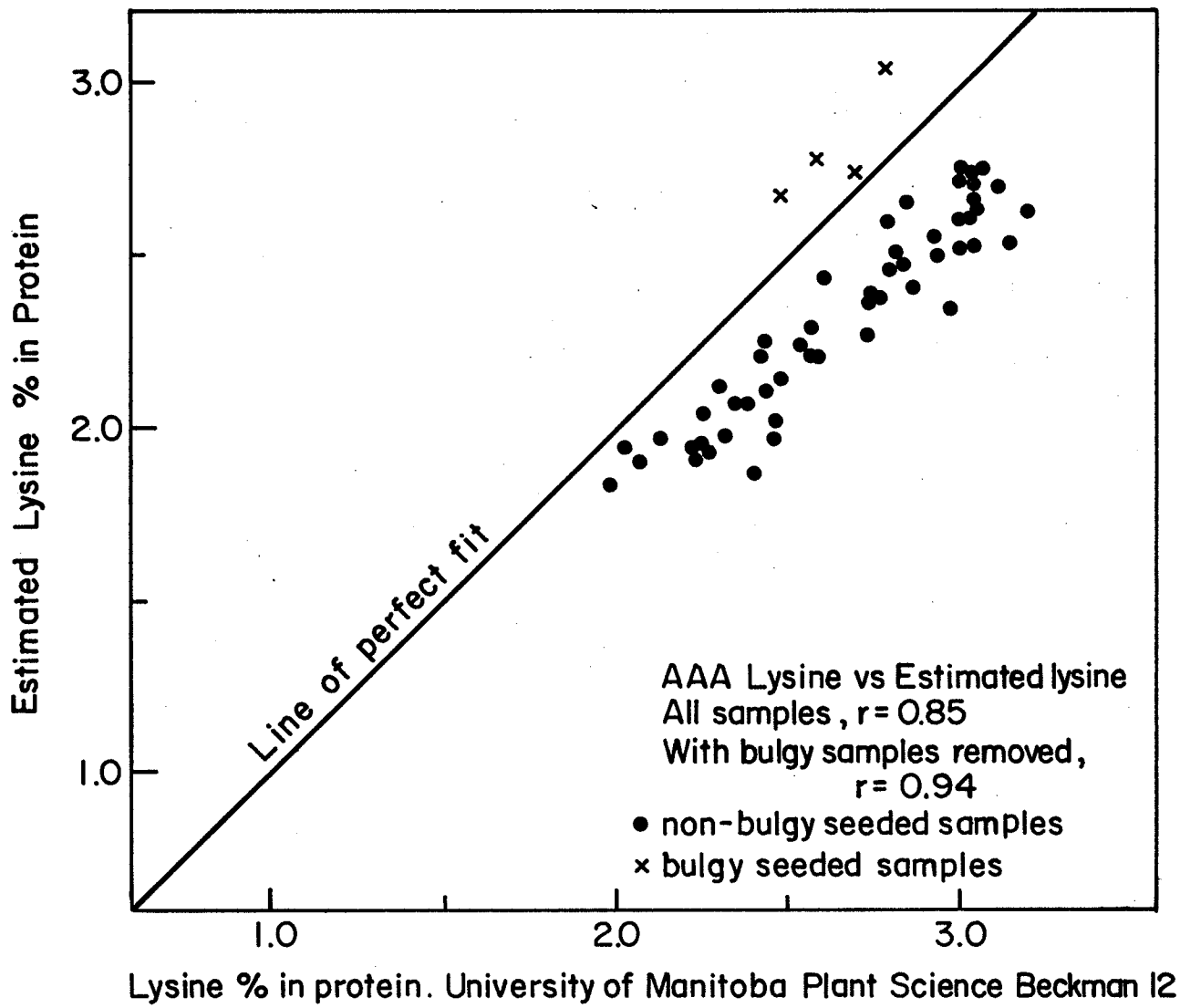

Figure 1.2. Comparison of estimated lysine with AAA lysine values from University of Manitoba Plant Science Beckman 121, using samples with a wide range of protein and lysine levels. Protein = N x 6.25.

TABLE 1.2. Estimated lysine and basic amino acids (as percent of protein, N x 6.25) in samples of the bulgy seeded lines (Q.50662 and Q.50687) compared with samples of CSH-1, P-721, and Q.50999.

Sample AAA#	Line	Estimated Lysine	U. of M. Plant Science Beckman 121		
			Lysine	Histidine	Arginine
111	Q.50662	2.75	2.70	2.17	4.36
112	Q.50662	3.05	2.77	2.08	4.16
142	Q.50662	2.68	2.44	1.93	3.83
113	Q.50687	2.79	2.58	2.10	4.21
101	CSH-1	1.95	2.03	2.28	3.75
109	P-721	2.67	2.86	2.14	4.58
121	Q.50999	2.22	2.54	2.22	4.31

it would appear that the ICRISAT Beckman was producing consistently lower lysine values than the University of Manitoba Plant Science Beckman.

To evaluate the relative accuracy in predicting lysine in contrasting environments, a division in the group of 52 samples was made. Those samples which had been grown in high nitrogen (HN) environments, with 150 kg, N/ha top dressed, contained a mean protein of 11.2 percent, with a range of 8.6 to 14.4 percent. Lysine values for these samples are shown in Figure 1.3. Again, a high correlation ($r = 0.95$) was found between estimated and AAA lysine values. Also, the demarkation between genetically high and normal lysine samples was quite distinct, whichever method of lysine evaluation was used.

Samples from the same lines, but grown without topdressed nitrogen (LN), are shown in Figure 1.4. These samples had a mean protein level of 7.6 percent, and ranged from 5.6 to 9.9 percent. In this set, the correlation between estimated and expected lysine was lower, $r = 0.88$. The lower values of both DBC and protein in these samples may have produced DBC/P ratios which were somewhat more variable than in the HN group. In this low protein set, the genetically normal lysine samples were higher in lysine while the genetically high lysine samples tended to retain the same lysine levels. This was true for both methods of lysine analysis. It would appear that genotypes had different lysine levels in the HN environment which were not expressed in the LN environment. Thus, there was a genotype by environment interaction between the normal and high lysine lines when grown in different environments. This interaction will be investigated in a subsequent manuscript.

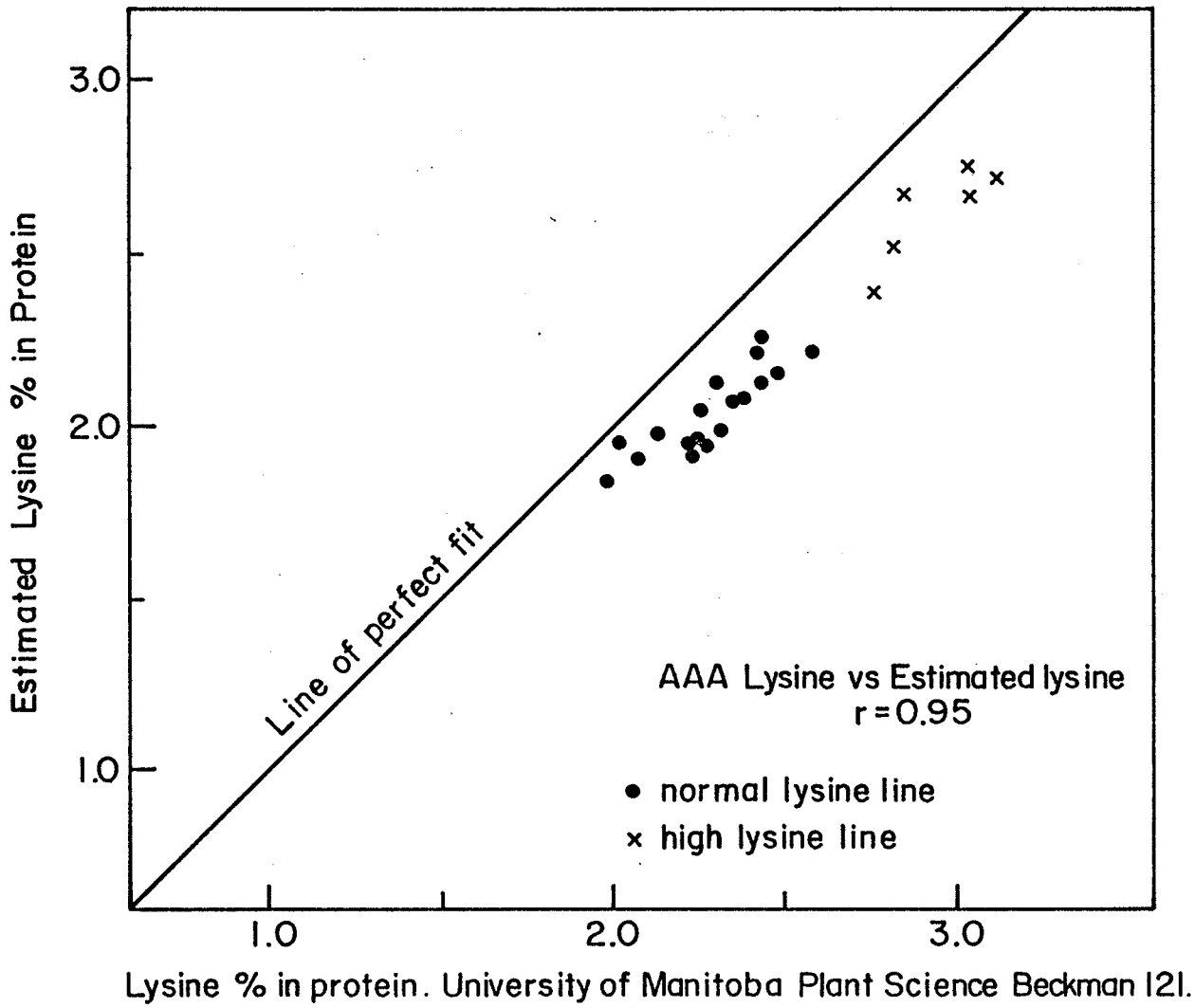

Figure 1.3. Comparison of estimated lysine with AAA lysine in samples from environments with 150 Kg/ha of top dressed nitrogen producing high protein values. Protein = N x 6.25.

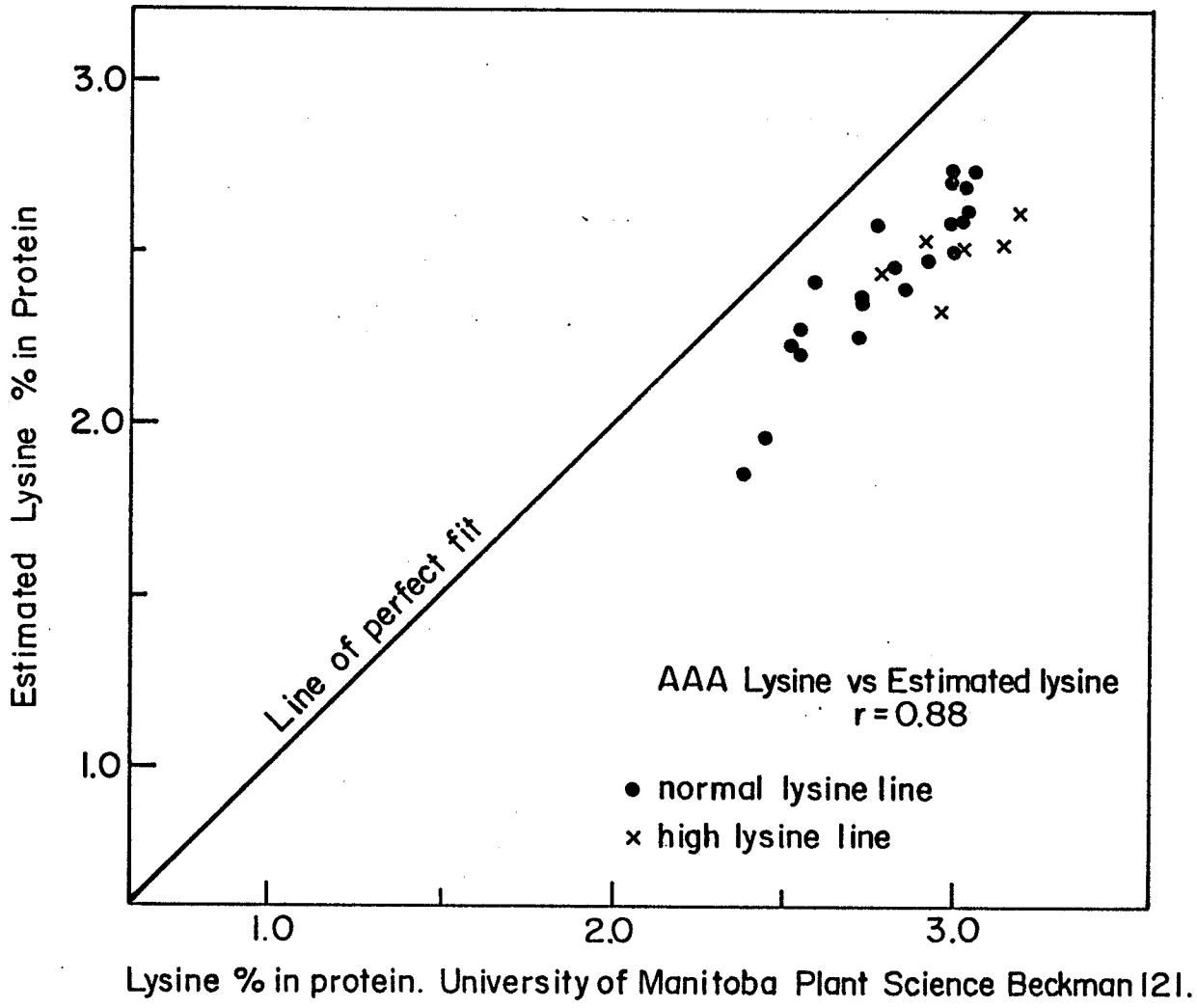

Figure 1.4. Comparison of estimated lysine with AAA lysine in samples from environments without nitrogen top dressing producing low protein values. Protein = N x 6.25.

The variation found in routine laboratory analysis of a hidden check sample is shown in Table 1.3. The mean protein of the checks from the different laboratory batches ranged from 10.16 to 10.51 percent and the mean DBC of the batches ranged from 31.8 to 33.0. The coefficient of variation, which included sampling as well as laboratory error, was 2.71 percent for protein and 3.22 percent for DBC. It was concluded that the laboratory variation associated with the protein and DBC values was within the acceptable limits.

Table 1.4a presents the estimated mean squares used in computing variances when the error mean square was subdivided into mean square terms associated with location in the field (F), sampling (S), and laboratory determination (D). Table 1.4b presents the magnitude of these variances as well as coefficients of variation associated with these components. The variation due to laboratory determination (σ_d^2) was relatively small, and was about equal for the HMB and LMR sites, both for protein and for DBC values. The size of σ_s^2, which is the variation due to taking 4 g samples of seed (100 - 200 seeds) from a well mixed plot sample, was surprisingly large, and was more important than the laboratory determination variation (σ_d^2) in the heterogeneous site. This indicated that seed-to-seed variation was importnat in 4 gram seed samples, and was greater at the more heterogeneous site. The field variation (σ_f^2) was greater in the more heterogeneous site, and was much more important than the other components of error variation for protein. For DBC values, the field variation was equal to or less than the other components in the more uniform site, but much more important in the more heterogeneous site.

TABLE 1.3. Error in laboratory estimation of protein and DBC values for hidden checks of a single seed lot of GPR-148.

Date Analyzed		Number	Mean	
			Protein (%)	DBC
1978	March	32	10.34	32.14
	May	9	10.51	33.00
	July	12	10.33	32.13
	October	34	10.16	32.17
1979	January	10	10.28	31.80
	February	8	10.19	33.00
	March	20	10.48	32.60
	Mean		10.32	32.31
	Coefficient of variation		2.71%	3.22%
	Standard deviation		.28	1.04

TABLE 1.4a. Estimated mean squares used in computing sources of variation in laboratory samples.

Source		df	Estimated Mean Squares
Genotypes	G	2	$\sigma_d^2 + 2\sigma_s^2 + 4\sigma_f^2 + 16\sigma_g^2$
Replications	R	3	$\sigma_d^2 + 2\sigma_s^2 + 4\sigma_f^2 + 12\sigma_r^2$
Field (GxR)	F	6	$\sigma_d^2 + 2\sigma_s^2 + 4\sigma_f^2$
Samples	S	12	$\sigma_d^2 + 2\sigma_s^2$
Determinations	D	24	σ_d^2

TABLE 1.4b. Comparison of Field (f), Sampling (s) and Determination (d) variances for protein and DBC values from 2 environments: a uniform site (HMB), and a heterogeneous site (LMR).

Site		Protein %		DBC	
		HMB	LMR	HMB	LMR
Means:		10.49	10.92	37.0	38.4
Variances:					
Field	σ_f^2	.102	.228	.476	4.430
Sample	σ_s^2	.015	.037	.406	2.260
Determination	σ_d^2	.020	.022	.828	.712
Coefficients of variance					
Field	F	6.47%	9.21%	9.56%	12.47%
Sample	S	2.12%	2.86%	4.42%	5.96%
Determination	D	1.36%	1.36%	2.45%	2.19%

CONCLUSIONS

Estimated lysine values, calculated from a prediction equation based on a dye binding capacity/protein and a protein term, appeared to be reasonably satisfactory in predicting actual lysine values (as percent of protein) from amino acid analysis. There was evidence to suggest, however, that lysine values from the University of Manitoba Plant Science Department Beckman 121, were consistently higher than values based on the ICRISAT Beckman 120C. It appeared that estimated lysine values were closely correlated with AAA lysine values in high protein samples ($r = .94$), and fairly closely in lower protein samples ($r = 0.88$). A genotype by environment interaction in lines with genetically different lysine levels could be detected by either method of lysine determination.

There appeared to be some lines in which lysine was not well estimated by the dye binding capacity/protein method. Therefore, lysine estimates should still be confirmed in samples of representative lines being tested.

Although the error associated with laboratory determination for protein and DBC was reasonably small, the variation found in taking 4 gram samples from well mixed seed lots was surprisingly large. This would indicate that an adequate sample size is important in order to minimize error variation in protein and DBC determinations.

MANUSCRIPT 2

ASSESSMENT OF SORGHUM LINES FROM THE HIGH LYSINE BREEDING PROJECT AT ICRISAT

INTRODUCTION

The high lysine breeding program of ICRISAT was initiated in 1973, based on the Ethiopian high lysine sources IS.11167 and IS.11758, which contain *hl*, a recessive gene for high lysine. These lines had approximately a 70 percent increase in lysine compared with normal lines, but had dented kernels and were reported to yield about 25 percent below comparable normal lysine lines (Singh and Axtell, 1973; Axtell *et al.*, 1978). Selections made at ICRISAT, derived from the *hl* sources, which had good agronomic appearance and plump seeds, were screened for high lysine using Udy dye binding values (Udy, 1971), adjusted for the protein percent in the sample. Although plump-seeded selections with high values were found in early generation selections, only a few lines expressing moderately high values were obtained from these selections in later generations. Thus, the initial results at ICRISAT from selection for high lysine, plump-seeded sorghums from the Ethiopian *hl* parents have been disappointing. Also, in spite of breeding efforts in several countries, no plump-seeded sorghum line which also contains the *hl* gene has yet been reported.

The second high lysine mutant, P-721, became available in 1975. This mutant has a reported 60% increase in lysine, and although the seeds were plump, they were 25 percent lighter than in the normal parent (Mohan, 1975). At ICRISAT, P-721 was first crossed with lines derived from the Ethiopian high lysine sources, and a substantial number of progenies from these crosses were found with DBC/P values equal to or greater than the P-721 parent. Axtell *et al.* (1979) have suggested that in appropriate crosses with P-721, it should be possible to find higher yielding progeny with high lysine levels by selecting for higher seed

numbers per head and increased number of heads per hectare. Breeding techniques involving intercrossing and selection have been used in the high lysine project at ICRISAT, in an attempt to improve both the agronomic performance and lysine level in sorghum for the Semi-Arid Tropics.

Because it was not known whether the seed denting and high lysine effects were controlled by closely linked genes or were acting as pleiotropic effects of the *hl* gene, an attempt was made to determine if the *hl* gene was present in any of the non-dented lines arising from the high lysine project at ICRISAT. Secondly, an evaluation was made of the effects of selection for high lysine (DBC/P ratios) in either inter-crosses derived from the Ethiopian high lysine sources, or in crosses with P-721.

MATERIALS AND METHODS

Single head selections which were being carried forward in the high lysine breeding project were grouped according to the DBC/P and protein values expressed during generations of selection. From 1500 F_6 and F_8 rows grown during the 1977 summer (hot, dry) season, 13 uniform lines which were representative of 5 groups were chosen: 2 lines from the high protein, high lysine group; 3 lines from the low protein, high lysine group, 3 lines from the unstable protein, high lysine group; 4 lines from the unstable lysine group; and one line expressing low protein and low lysine values during generations of selection. These selections, which were entered into stability study 1, all had one of the Ethiopian *hl* parents, IS.11758 or IS.11167, in their pedigrees. Six selected entries were derived from straight crosses with a Purdue Population (PP) selection, and the remaining seven entries were either from back-crosses or intercrosses involving selections from the Nebraska Population (NP), Purdue Population or Maunders Yellow Population (MY). Except for PP_9, which included crosses to the *hl* parents, none of these populations possessed any known high lysine genes. Another three entries were checks: CSH-1 is a popular Indian hybrid; P-721 and IS.11758 are both high lysine mutants. More details on these lines are provided in Table A-1. Protein and estimated lysine values of each of the entries during the last generation of single head selection were compared with the means of each line tested in 12 environments during the 1977 cool, dry (Rabi) season. The use of dye binding capacity and protein (DBC/P) values in producing estimates of lysine percent in protein is described in Manscript 1, page 37, and a description of the 12 environ-

ments used in testing the entries is found in Manuscript 3, page 73.

To test for the presence of the *hl* gene, the lines in stability study 1 were crossed with IS.11758. Three F_1 plants in each cross were grown adjacent to the parents, during the 1978 cool, dry season, with 50 cm between both plants and rows. Each plant produced up to 4 culms, then excess tillers were removed. Fertilizer at the rate of 60 kg N/ha and 26 Kg of P/ha was applied at planting with 150 kg N/ha subsequently applied in two top-dress applications. Heads were not selfed because of the possible influence of bagging, both in causing plump seeds to be classed as non-plump, and in influencing protein and estimated lysine values. Instead, adjustment for outcrossing was made by using two lines with recessive endosperm markers. Samples were obtained by taking three to ten heads from each cross, and counting four hundred F_2 seeds from each head. These seeds were classified into plump or dented, or in the case of two crosses, into plump, "bulgy," and dented seeds. Chi-square tests were made to test the observed segregation against genetic hypotheses. Chemical analyses were carried out on the classified groups of seed for protein percent and estimated lysine values.

The second high lysine mutant, P-721, was crossed with a large number of single head selections from the high lysine breeding program (known as *hl*-derived selections) during the 1975 cool, dry and the 1976 hot, dry seasons. At the same time, *hl*-derived selections were intercrossed with each other. Two sets of six crosses were chosen in order to evaluate the effect of the source of high lysine, as well as to evaluate the trade-offs between selecting for either grain weight per head or for DBC/P values. Table 2-1 presents the procedure used in

TABLE 2.1. Selection procedure followed to develop four groups of lines from P-721 or *hl* -derived parents, with progeny selected for either DBC/P, or for grain weight per head.

Season	Generation	Procedure
1975 cool, dry and 1976 hot, dry	Parents crossed	2 sets crossed - (a) P-721 x *hl*-derived lines and (b) *hl*-derived lines intercrossed. Six crosses in each set.
1976 warm, rainy	F_1	Representative heads from each cross advanced
1977 hot, dry	F_2	10-30 heads selected in the field from each of the 2 sets of crosses. In each cross, 2 heads with the highest DBC/P ratio and 2 heads with the highest grain weight per head were chosen, resulting in 4 groups of selections.
1977 warm, rainy	F_3	Selections were grown in 3-row plots, with blocking according to cross. 10 heads were chosen for agronomic performance in each plot. In each cross, the 2 heads with highest DBC/P values, and the 2 heads with highest grain weight, were chosen from the progenies of the heads previously selected for that trait.
1977-78 cool, dry	F_4	Performance trial of the selected lines (8 or 12 lines in each of the 4 groups) plus 3 checks in 3-row plots with 2 replications in 2 locations in India (9^{o}N and 18^{o}N lat.). The centre row (2.5 m) harvested from each plot.

selecting the lines from these 2 sets of crosses. One of the parents in each of the *hl*-derived intercrosses was also used as the parent in crossing with P-721, in order to reduce the effects of different *hl*-derived parents in the comparisons. Four F_4 lines from each of the 2 sets of 6 crosses were selected for replicated testing. In two of the intercrosses between the *hl*-derived lines, it appeared that a mistake in crossing had occurred, and the lines from these crosses were therefore not included in the comparison. T-tests, computed on the means of either 8 or 12 F_4 lines grown with two replications at two locations were used to compare the four groups, which differed by either the high lysine source or for the trait selected. Comparisons were made for protein percent (N x 6.25, Technicon Method, Mitcheson and Stowell, 1969), estimated lysine, grain yield, 100 kernel weight, and vitreousness score.

RESULTS AND DISCUSSION

In Table 2.2, the mean estimated lysine and protein values of the *hl*-derived lines in stability study 1 from 12 environments are compared with the values found in single head selections in the 1976 warm, rainy season, the last generation before bulking. The disappointingly low frequency of high DBC/P plants in the breeding material, mentioned earlier, meant that several selections in the high lysine groups had lower estimated lysine values than did the P-721 check.

Of the 13 *hl*-derived selections, only the two in the high protein, high lysine group--Q.50662 and Q.50687--maintained high protein and high estimated lysine when tested across environments. All the selections made for high estimated lysine, in the low or unstable protein groups, produced lower estimated lysine levels, comparable with the CSH-1 hybrid check, when the lines were tested across environments. Q.50738, and Q.52155, which were selected for low lysine, produced higher, (normal) estimated lysine values when tested across environments. Likewise, with the exception of Q.50662 and Q.50687, the range in protein which existed in the individual head selections decreased greatly when the lines were tested over environments.

The seeds of Q.50662, and Q.50687, the two lines that appeared to maintain high lysine, possessed a characteristic bulge opposite the embryo, and will be referred to as the bulgy lines. A comparison of these two lines with P-721 high lysine mutant and the hybrid check CSH-1 is made in Table 2.3. The two bulgy lines were slightly later in flowering than P-721 or CSH-1. Grain yields of these two lines are similar to that of P-721, but were much lower than

TABLE 2.2. Protein percent, and estimated lysine (as percent of protein) in *hl*-derived selections which were used in stability study 1.

Early generation grouping and line number	F_5 and F_7 heads selected in 1976 warm wet season		Mean of lines in 12 environments 1977 cool dry	
	Prot.	Est. lys.	Prot.	Est. lys.
High protein, high lysine				
Q.50662	14.1	2.61	13.5	2.57
Q.50687	13.2	2.40	12.7	2.55
Low protein, high lysine				
Q.50890	8.7	2.34	9.5	2.24
Q.50902	7.3	2.30	10.0	2.12
Q.50999	8.1	2.35	10.9	2.16
Unstable protein, high lysine				
Q.51008	8.1	2.30	10.9	2.12
Q.51056	7.3	2.37	10.4	2.06
Q.51234	7.1	2.44	10.2	2.16
Unstable lysine				
Q.52155	10.9	1.81	10.7	2.07
Q.50738	12.1	1.79	10.4	2.12
Q.50922	9.4	2.26	9.8	2.11
Q.50699	7.8	2.69	9.3	2.16
Low protein, low lysine				
Q.53220	6.7	2.12	9.3	2.13
Checks	replicated means			
P-721	12.0	2.60	11.48	2.54
IS.11758	-	-	13.46	3.24
CSH-1	8.5	2.09	8.14	2.17
		* $S\bar{x}$	0.11	0.02

* $S\bar{x}$ = standard error of a genotype mean at 0.05 probability level.

TABLE 2.3. Peformance of the bulgy lines (Q.50662 and Q.50687), compared with P-721 and CSH-1. Means of lines in 12 cool dry season environments.

Character	Q.50662	Q.50687	P-721	CSH-1	$S\bar{x}$
Days to 50% flowering	79.0	77.0	74.4	72.2	.05
Yield (Kg/ha)	2125	1904	2173	4378	91
100 kernel wt (g)	2.08	2.21	2.39	3.56	.02
Vitreousness*	4.81	3.56	1.26	3.84	.08
Lysine (% in Protein)**					
Estimated	2.70	2.77	2.82	1.91	
Beckman AAA	2.58	2.58	2.73	1.86	
Seed Components***					
Pericarp %	17.3	15.8	12.6	8.6	
Embryo %	8.4	8.3	10.3	6.8	
Endosperm %	74.3	75.9	77.2	84.6	

*Endosperm vitreous score. 1 = completely floury, 9 = completely vitreous.

**Lysine values are means of two determinations from plots in two replications in low management black soil (vertisol) in cool dry season, with 150 kg N/ha top dressed. Values for Q.50687 are from a single determination.

***Seed component samples from single plots grown in low management black soil (vertisol) in cool dry season, with 150 kg N/ha top dressed.

$S\bar{x}$ = Standard error of a genotype mean.

the CSH-1 hybrid. Kernel weight was lower in the bulgy lines than in the P-721 mutant, and much lower than in CSH-1. The endosperms of the bulgy lines had approximately the same vitreousness scores as CSH-1 but the level of vitreousness was quite variable from seed to seed, and some of the most vitreous endosperms had small air spaces or voids.

The two bulgy lines had protein values (Table 2.2) close or equal to that of the Ethiopian *hl* mutant IS.11758 and above that of the P-271 high lysine mutant. Estimated lysine values (Table 2.2) indicated that the lysine values of the bulgy lines might be equal to, or very slightly higher than in the P-721 mutant. However, comparisons of estimated lysine with Beckman amino acid lysine values in a very limited number of samples (Table 2.3) indicated that the lysine in both bulgy lines was lower than in P-721. That the bulgy lines have a higher lysine level than normal varieties, but lower than that of P-721, is further confirmed by the results of the comparison of estimated *vs* Beckman 121 lysine values involving these lines (Manuscript 1).

Since the embryo of sorghum has been found to contain 5.42 percent lysine in protein (Shoup *et al.*, 1969), the possibility of a large proportion of embryo in the small seeds of the bulgy lines causing the high lysine effect was investigated. Seed components shown in Table 2.3 indicate that the proportion of pericarp was slightly higher in the bulgy lines which was likely due to smaller seed size. However, the embryo:endosperm relationship was similar for P-721 and the bulgy lines, indicating that the higher lysine in these bulgy lines was likely to be produced by the endosperm, rather than the embryo.

In order to determine if the *hl* gene was present in any of the *hl*-derived lines in stability study 1, test-crosses were made to IS.11758, a homozygous source of the *hl* gene. Three low lysine lines were crossed similarly to provide checks. F_2 seeds were obtained for all lines in stability study 1 except for Q.50738, and Q.50999. F_2 seeds (Table 2.4) can be classified into two different ratios. The normal lysine checks, as well as all *hl*-derived lines except the two bulgy lines, produced F_2 ratios close to 3:1, plump:dented. This ratio is expected in the normal lysine checks. In the *hl*-derived lines that produced F_2 ratios of 3:1 plump:dented it appeared that the *hl* gene had been lost. The greater than expected numbers of plump seeds in crosses with Q.50890, RS_1 x VGC, and Q.50902 may have been the result of higher levels of outcrossing in these lines. If denting and high lysine were closely linked or conditioned by the same *hl* gene, then the *hl* gene would have been lost by selecting plump seeded segregates in the high lysine breeding program. Any hypothesis involving modifier effects producing plump seeds in the presence of the *hl* gene can be ruled out because of the fairly close fit to a 3:1 ratio observed in these lines. All significant deviations were in the direction of an excess of plump grains, which did not indicate the retention of the *hl* gene in these lines.

In order to simplify classification, segregating F_2 seeds from testcrosses with the two bulgy lines Q.50662 and Q.50687 which were either dented or bulgy in appearance, were classed as dented/bulgy. F_2 ratios from both Q.50662 and Q.50687 testcrosses were close to a ratio of 27 plump:37 dented/bulgy. This ratio is expected when three recessive genes are segregating independently, and the occurrence

TABLE 2.4. F_2 ratios of plump:dented, or plump:dented / bulgy seeds of *hl*-derived lines from stability study 1, testcrossed with IS.11758.

♀ Parent	No. of heads Counted	Expected/		Observed*		X^2	P(b)
		Plump	Dented	Plump	Dented		
GPR-148 (Check)	6	1800	600	1830	570	1.0	.25
RS_1 X VGC (Check)	8	2400	800	2466	734	7.3	.005
BP-53 (Check)	4	1200	400	1194	406	.12	.75
Q.50890	8	2400	800	2535	665	30.3	.0001
Q.50902	3	900	300	941	259	7.5	.005
Q.51008	9	2700	900	2789	814	10.9	.001
Q.51056	10	3000	1000	3059	941	4.6	.025
Q.51234	5	1500	500	1519	481	.96	.25
Q.53220	9	2700	900	2764	836	6.0	.01
Q.50922	5	1500	500	1526	474	1.8	.10
Q.52155	9	2700	900	2678	922	.72	.25
Q.50699	3	900	300	888	312	.64	.25
			(Dented/ Bulgy)@		(Dented/ Bulgy)		
Q.50662	8	1350	1850	1414	1786	5.25	.01
Q.50687	7	1181	1619	1151	1649	1.32	.25

/ Expected ratios. 3 plump:1 dented.

@ 27 plump:37 (dented/bulgy), assuming 2 recessive "bulgy" genes from ♀ parent plus the *hl* gene from the tester parent.

* Adjusted for 1 percent outcrossing.

(b) P = probability of deviation being due to chance.

of a recessive homozygote at any one of these three loci can produce the bulgy or the dented phenotype. Since one recessive gene (*hl*) came from the IS.11758 parent, the other two recessive genes must have come from the bulgy parent.

Protein percent and estimated lysine values from samples of bulgy, dented or plump F_2 seed as well as the parental seed are shown in Table 2.5. It is clear that the bulgy seed samples in the F_2 test-crossed seed from both bulgy lines produced estimated lysine values equal to the estimated lysine value in the bulgy parent lines. Thus, it appeared that the genes which condition the bulgy phenotype also produced the higher estimated lysine values in these two lines. The existence of plump, F_2 seed with low estimated lysine levels rules out the possibility that the *hl* gene together with bulgy modifiers produced the high lysine, bulgy phenotypes in the two bulgy lines.

Although the two bulgy lines originated from different crosses--Q.50662 came from (73PP_9R x IS.4562) x 73PP_9R(PP_3R x IS.11167), and Q.50687 from 73PP_9R x IS.11167)--the crosses had PP_9R in common. It may be that the bulgy genes originated from this population. Although PP_9R contained the *hl* gene, the above evidence suggested that the bulgy genes were independent of the *hl* gene. This may be somewhat similar to the high lysine system in maize reported by Choe and Zuber (1976), which was produced by recurrent selection, and was independent of the opaque or floury (o_2 or fl_2) high lysine genes.

There was also evidence that IS.11758 possessed a high protein genotypic component which was independent of the *hl* gene. In test-crosses with the 3 normal lysine lines GPR-148, Q.50890, and Q.53220,

TABLE 2.5. Protein percent, and estimated lysine (percent in protein) in plump, dented, or bulgy F_2 seed from testcrosses with IS.11758.

Cross or parent	No. of heads	Plump		Dented		Bulgy	
		Prot.	Est.lys.	Prot.	Est.lys.	Prot.	Est.lys.
		Crosses with bulgy lines					
Q.50662 x IS.11758	8	15.7	1.73	17.2	2.93	16.5	2.51
Q.50662	6					17.1	2.62
IS.11758	4			16.2	3.20		
Q.50687 x IS.11758	7	13.3	1.86	16.0	2.80	14.6	2.75
Q.50687	3					17.9	2.63
IS.11758	4			16.8	3.26		
		Crosses with normal lysine lines					
GPR-148 x IS.11748	6	14.0	1.94	15.8	3.03		
GPR-148	6	10.6	1.94				
IS.11758	4			15.5	3.22		
Q.50890 x IS.11758	8	13.2	1.94	15.15	3.12		
Q.50890	11	12.0	1.90				
IS.11758	5			16.2	3.24		
Q.53220 x IS.11758	9	13.5	1.92	15.0	3.12		
Q.53220	9	11.9	1.95				
IS.11758	6			16.1	3.15		

which have been shown not to contain the *hl* gene, the plump F_2 grain samples were 1.2 to 3.4 percent higher in protein than the normal lysine parents (Table 2.5). If the *hl* gene was the only source of higher protein, the plump-seeded F_2 grain samples, in which the *hl* gene was not expressed, would produce protein percentage equal to the normal lysine parents. The observed increases in protein in plump F_2 seed samples over the testcross parent, must be due to genetic effects from IS.11758 which are independent of the *hl* gene.

The effect of the incorporation of the P-721 high lysine source is shown in Table 2.6, in which the performance of a set of crosses between P-721 and *hl*-derived lines was compared with a set of *hl*-derived lines intercrossed with each other. Selections were made in both sets either for high DBC/P ratios or grain weight per head. Only the P-721 x *hl*-derived set of crosses in which selection was made for DBC/P (group 2), produced lines with high estimated lysine values, with a mean approximately equal to P-721. Lines in group 2 also produced a mean yield equal to that of P-721. However, the production of high estimated lysine in group 2 lines was accompanied by a reduction both in seed size and in endosperm vitreousness. In contrast to group 2, selection for high DBC/P ratios among the *hl*-derived intercrosses (group 4) was not effective in increasing estimated lysine. Surprisingly, selection for high DBC/P ratio did not appear to change protein levels in either group of crosses, nor did selection for grain weight per head produce higher yields. However, the set of *hl*-derived intercrosses (groups 3 and 4) did produce higher mean yields than did the set of P-721 crosses (groups 1 and 2).

TABLE 2.6. Comparison of four groups of F_4 lines arising from *hl*-derived lines crossed with P-721, or intercrossed with another *hl*-derived line. F_2 and F_3 heads were selected for either high DBC/P ratios or for grain weight per head (grain wt).

Set and trait selected	Group	Protein %	Est. Lys. (% in Prot.)	Yield (kg/ha)	100 Grain wt (g)	Vitreousness score@
P-721 x *hl*-derived						
Grain wt	1	13.4	1.93	2686	2.56	2.84
DBC/P	2	12.5	2.56	2843	2.09	1.69
t-test	1 vs. 2	NS	**	NS	**	**
hl-derived intercrosses						
Grain wt	3	12.7	1.92	3200	2.37	2.69
DBC/P	4	12.4	2.02	3364	2.40	2.67
t-test	3 vs. 4	NS	NS	NS	NS	NS
P-721 x *hl*-derived	1 + 2	13.0	2.23	2764	2.32	2.76
hl-derived intercrosses	3 + 4	12.5	1.97	3282	2.38	2.18
t-test	(1 + 2) vs.(3 + 4)	**	*	*	NS	NS
Checks						
P-721		13.5	2.61	2665	2.38	1.00
CSH-1		10.8	1.89	4192	3.44	3.11

@ Score for endosperm vitreousness 1 = floury, 9 = completely vitreous.

* Probability of difference being due to chance < .05.

** Probability of difference being due to chance < .01.

Thus it appeared that intercrossing and selection among the *hl*-derived lines was not successful in producing high lysine lines, but that high lysine lines could be recovered from crosses with P-721. In spite of selection for good agronomic appearance along with selection for DBC/P ratios, the mean yield of P-721 crosses (group 4) was not increased above that of the P-721 parent. The lack of response to selection for head weight was also disappointing. This may have been due to genotype by season interaction, as it was observed that some F_3 selections having very large heads during the 1977 warm, wet season produced poorly exerted F_4 heads when tested in the 1977 hot, dry season.

In the high lysine breeding program, which involved several hundred crosses between P-721 and *hl*-derived lines, progenies with lysine levels equal to that of the P-721 parent were found, but with agronomic appearance much below that of CSH-1. This may be partially due to the *hl*-derived parents, which had been intensively selected for high DBC/P ratios but the agronomic appearance of which was not very impressive.

CONCLUSIONS

It was apparent that the *hl* gene was not present in the *hl*-derived plump-seeded lines which were selected from the high lysine breeding material at ICRISAT. There was a strong indication that the denting and high lysine characteristics were inherited together; both characters appeared to be controlled by the same *hl* gene. Work with the *hl* gene at Purdue University had similar implications (Axtell *et al.*, 1978).

Selection for high dye binding capacity/protein values had produced two lines with moderately high lysine and a bulgy appearance of the kernels. There was evidence that two independent recessive genes controlled both the improved lysine and the bulgy appearance of these kernels.

The large differences in lysine and protein that can occur when advanced generation individual head selections are compared with more reliable values of progeny lines tested over environments, are likely due to environmental effects on the single plant. In the high lysine breeding program at ICRISAT, selection of plump-seeded segregates from crosses with the dented Ethiopian high lysine lines, followed by selection for protein and lysine, may have resulted in selection for environmentally induced high lysine heads. It would appear to be important to confirm lysine and protein levels by growing progeny of selected heads on a plot basis, with either interlarded checks, or replications to measure the error variation.

Further intercrossing and selection among the *hl*-derived lines were not effective in increasing lysine levels. However, high lysine selections from crosses with the P-721 high lysine mutant were readily

obtained. These selections had low kernel weight, and floury endosperms, similar to their P-721 parent. Also, yields in F_4 lines from crosses with P-721 were lower than in F_4 lines from intercrosses with the *hl*-derived lines.

MANUSCRIPT 3

RESPONSE OF PROTEIN, ESTIMATED LYSINE AND GRAIN YIELD OF SORGHUM TO SOME ENVIRONMENTAL EFFECTS IN THE SEMI-ARID TROPICS

INTRODUCTION

The importance of location and seasonal effects on protein and lysine levels have been well established in grain sorghum (Deosthale and Mohan, 1970; Worker and Ruckman, 1968). However, it has been difficult to relate variation in crop response to environmental factors such as rainfall, temperature, soil type and fertility level (Eberhart and Russell, 1966, Binswanger and Barah, 1979).

In the high lysine sorghum breeding program at ICRISAT, different locations and seasons were used to select and advance generations of segregating progenies. Large fluctuations in estimated lysine and protein levels were found in these progenies from one generation to the next. The location and seasonal differences may have been a factor in the failure to recover the high lysine effect in all but a few lines derived from these progenies. Such environmental differences are also likely to affect nutritional quality of sorghum grain coming from farmers' fields.

This manuscript presents information derived from two factorial experiments in which the environmental variation was divided into a number of factors. The relative importance of the influence of each factor on levels of protein, estimated lysine and grain yield, as well as on days to flowering, kernel weight and endosperm vitreousness was examined, and the degree of interaction between the factors was assessed. In addition, an examination was made of the interrelationships between protein, estimated lysine and grain yield in these environments.

MATERIALS AND METHODS

Two factorial split-plot experiments were carried out to investigate the responses of sorghum to a range of environments typical of the semi-arid tropics. Factors in these experiments are outlined in Table 3.1, and a more complete description follows.

The two-season experiment was grown during both the 1977 cool, dry (Rabi) and 1978 warm-wet (Kharif) seasons. In each season, trials were planted in 4 different areas on the ICRISAT research farm, which included two levels of management, and two different soil types. The high management area (HM) had precision fields, graded to a 0.4 percent slope, which had received an average of 26 kg/ha of P and 100 to 200 kg/ha of N in each of the previous 5 - 6 seasons. During the seasons when these trials were grown, HM areas received 48 and 38 kg N/ha in the 1977 cool dry, and 1978 warm wet seasons respectively, and 26 kg of P/ha in both seasons. This fertilizer was banded into the ridge at planting. Low management (LM) areas were meant to approximate farmers' conditions. These areas were either unlevelled, or had minimal levelling, and had received a maximum fertilizer application of 20 kg/ha of N and 9 kg of P/ha in past seasons. This rate of N and P was also applied at planting during the seasons when these trials were grown. Each management area included both black swelling clay vertisols (B) and red sandy loam alfisols (R). These soil types are typical of large areas of the semi-arid tropics.

Each trial had two nitrogen treatments as main plots, which were replicated four times. The low nitrogen (LN) treatment received a single nitrogen application at planting, which varied according to the

TABLE 3.1. Environmental factors in the two-season and irrigation experiments.

Factor	Levels	Description of Levels	
		Two-season Experiment	
Season (Y)	2	1977 Cool-dry Rabi (7R)	1978 Warm-wet Kharif (8K)
Management (M)	2	High - research (HM)	Low - farmers' (LM)
Soil (S)	2	Black vertisol (B)	Red - alfisol (R)
Nitrogen (N)	2	High nitrogen (HN) basal + 2 top dress applications.	Low nitrogen (LN) basal application only.
Genotypes (G)	18	13 *hl*-derived entries, P-721, CSH-1, and 3 normal lysine check varieties.	
		Irrigation Experiment	
Soil (S)	2	Black vertisol (B)	Red alfisol (R)
Irrigation (I)	2	Fully irrigated (W)	Restricted irrigation (D)
Nitrogen (N)	2	High nitrogen (HN)	Low nitrogen (LN)
Genotypes (G)	22	18 Genotypes common to 2-season experiment , IS.11758, 2 other check varieties and one *hl*-derived line.	

management level and season (rates were set by the ICRISAT Farm Research Committee). Applications of 38 and 48 kg N/ha were made during the 1977 cool dry and 1978 warm wet seasons, respectively, on the high management areas, and 20 kg N/ha was applied on the low management areas during both seasons. The high nitrogen (HN) treatment received the same basal N application as did the LN treatment, but two additional top-dress N applications were made. An application of 50 kg/ha was made at panicle initiation stage, and 100 kg N/ha was added at the boot leaf stage. The HN and LN treatments provided 2 environments at each site.

Each genotype was planted in a sub-plot, consisting of 3 rows, 4 metres long. The genotypes were part of a set referred to as stability study I (Manuscript 2). Three of these genotypes, *viz.* P-721, and two 'bulgy' lines - Q.50662 and Q.50687 - had high and moderately high lysine levels. The other entries were made up of eleven lines from the high lysine program at ICRISAT, and four checks, consisting of a hybrid - CSH-1 - and three released or promising varieties. These fifteen entries all had normal lysine levels (Table A.1).

Abbreviations will be used when referring to the different environments. For example, 7RHMB-HN will refer to the plots grown in the 1977 cool dry (Rabi) season under high management conditions on a black vertisol, with 150 kg N/ha received in two top dress applications which followed 48 kg N/ha at planting.

Regular irrigation, every three weeks and every 12 days was applied to black and red soil trials, respectively, during the cool dry season, and no moisture stress was visible in these trials. Trials planted during the warm-wet season were rainfed, and short periods of moisture stress

developed on the shallow, lighter-textured red soils between rains.

To compare the results of this factorial set of trials with other locations, similar trials were laid out during the 1978 warm, wet season on a fine sandy loam aridisol at Hissar (HIS) in north India (29°N lat), and on a vertisol at Parbhani (PAR) in west-central India (19°N lat), and during the hot, dry summer (S) season on red sandy loam alfisol at Bhavanisagar in south India (11°N lat).

At all sites plant population was 120,000 plants/ha, in ridges 75 cm apart at ICRISAT, and in 45, 50 and 50 cm row-spacing at Hissar, Parbhani and Bhavanisagar respectively, in accordance with local practice.

In every trial, granular carbofuran insecticide was applied below the seed at planting to control sorghum shootfly (*Artherigona varia soccata*). Pesticides were applied as necessary against stem borer (*Chilo partellus*), sorghum midge (*Contrinaria Sorghicola*) and ear head bugs (*Calichoris* ssp). Dithane M-35 fungicide was sprayed on the heads weekly for three weeks following flowering, at the ICRISAT sites during the 1978 warm wet season, in an attempt to reduce the incidence of grain mold.

Plants from three metres in the centre row of each sub-plot were used for agronomic and biochemical evaluations. During the season, evaluations were made for pest incidence and the days to 50 percent flowering was recorded for each plot. Data on numbers of plants, numbers of heads, and grain yield per sub-plot were multiplied by the appropriate factor and expressed on a per hectare basis. One hundred kernel weights were calculated on 300 kernels per sub-plot in the cool

dry season and on 100 kernels per sub-plot in the warm wet season trials. Endosperm vitreousness was scored on 30 kernels per sub-plot in the cool dry season trials, and on 10 kernels per sub-plot in the warm wet season trials. Floury endosperms were given a score of 1, ranging to a score of 9 for completely vitreous endosperms. Technicon protein (Mitcheson and Stowell, 1969) and DBC (Udy, 1971) values were determined on four grams of ground sample, which was taken from a well mixed sub-plot sample, three to six months after harvest. Moisture in the samples was regularly determined and was found to vary from 8.5 to 9.8 percent; no adjustment was made for moisture.

Estimated lysine values (as percent of protein) were obtained from a prediction equation (Manuscript 1), using protein and DBC/P values. Other variables were then calculated by transformation. These were:

$$\text{Protein in kg/ha} = \frac{\text{Protein \% x grain yield (Kg/ha)}}{100} \qquad (1)$$

Estimated lysine as percent of a sample =

$$\frac{\text{Estimated lysine (as \% of protein) x protein \%}}{100} \qquad (2)$$

Number of kernels per head =

$$\frac{100 \times \frac{\text{grain yield (kg/ha)}}{\text{No. of heads/ha}}}{\text{100 kernel weight (g)}} \qquad (3)$$

Protein per kernel (g) =

$$\frac{\text{Protein \% x 100 kernel wt (g)}}{100} \qquad (4)$$

(Environmental means for protein per kernel were calculated, but statistical analysis was not carried out for this variable.)

Individual trials were first analyzed statistically in a split-plot design with genotypes as sub-plots and nitrogen fertilizer levels as main plots. Error variances of trials were then compared using Bartlett's test (Snedecor and Cochran, 1967) and an attempt was made to correct for heterogeneity of error terms by appropriate transformations.

In the analysis of combined sites (2-season experiment), the genotype (G) factor was considered to be a random effect, while all other factors were fixed effects. Two error terms were calculated by pooling the appropriate higher order interaction components. The main plot error term, σ_a^2 was contained in the mean squares of main effect components for nitrogen (N), soil types (S), management (M) and season (Y), as well as in interaction components involving these main effects. The split plot error term, σ_b^2, was contained in the mean squares for genotypes (G) as well as in interactions between genotypes and Y, M, S, and N. The expected values of mean squares in this analysis are shown in Table A.2. Calculations of F tests, using the appropriate mean square terms, and of variances from the expected mean squares formulae, could then be made.

The irrigation experiment was conducted during the 1977 cool dry season, on the two soil types in the high management areas of the ICRISAT farm. The fully irrigated sites, called BW and RW, were common to the 7RHMB and 7RHMR sites in the 2-season experiment (Table 3.1). In addition, 2 sites with restricted irrigation - BD and RD - were laid out next to the irrigated sites in the black and red soils. In the red soil site with restricted irrigation (RD), regular irrigation was con-

tinued up to five weeks, then stopped. The moisture stress which developed was relieved by a single irrigation, followed by a light rain at flowering. Progressive moisture stress developed during seed filling. The black soil site with restricted irrigation (BD) received only a single irrigation to insure uniform germination. Except for a few light showers, this crop grew on stored soil moisture. The irrigation schedules for the fully irrigated red (RW) and black (BW) soil sites were previously described (page 74). The same agronomic and chemical evaluations were made on this irrigation set as were earlier described for the two-season experiment. The same statistical analysis was also used, except that management (M) and seasons (Y) were not included as factors. In addition to the 18 genotypes in the two-season experiment, four additional genotypes were included in the irrigation experiment. These included three normal lysine sorghum lines, and the Ethiopian high lysine mutant IS.11758, which were adapted only to the short days of the cool dry season (Table A.1). Of the 22 lines in this experiment, four entries had high or moderately high lysine levels.

The relative importance of genotypic, environmental and genotype-by-environment variances was found in the two-season experiment for protein percent, estimated lysine, and grain yield. The four environmental factors, *viz.* season (Y), management (M), soil (S) and nitrogen (N) and their interactions were pooled into an environment component (E) while interactions between genotypes (G) and the four environment factors were pooled into a G x E component.

In both the two-season experiment and the wet-dry irrigation

experiment, combined sets of high lysine and normal lysine genotypes were used. To determine what effects, if any, the inclusion of high lysine lines had on the environmental response of these sets, variances for estimated lysine were calculated for each of the environmental factors and for their interactions in both a normal and a high lysine subset as well as in the combined set forming the irrigation experiment.

At the end of grain filling, soil samples were taken from the top 90 cm, using 16 cores to sample a site. Samples were dried, ground, and analyzed in the ICRISAT laboratory for a number of soil chemical properties. Available phosphorus, exchangeable potassium, exchangeable sodium, conductivity and pH measurements were carried out as described by Black *et al.* (1965). Available nitrogen was determined by the alkaline permanganate method (Subbiah *et al.*, 1956). Although this available nitrogen evaluation may not reliably determine actual nitrogen availability in a given soil, it was used in this case only to detect differences useful in making comparisons of means of two soils or soil treatments.

Weekly records of meteorological data during the time the trials were growing are illustrated in Figures 3.1 and 3.2. At ICRISAT centre, the 1977 cool dry season was characterized by steadily declining minimum temperatures during vegetative growth, down to 12°C, with a rapid rise during seed filling. Relative humidity remained low and rainfall was restricted to a few showers during planting and flowering. The 1978 warm wet season had temperatures that were most constant, with a narrower day-night spread, high relative humidity, and adequate rain

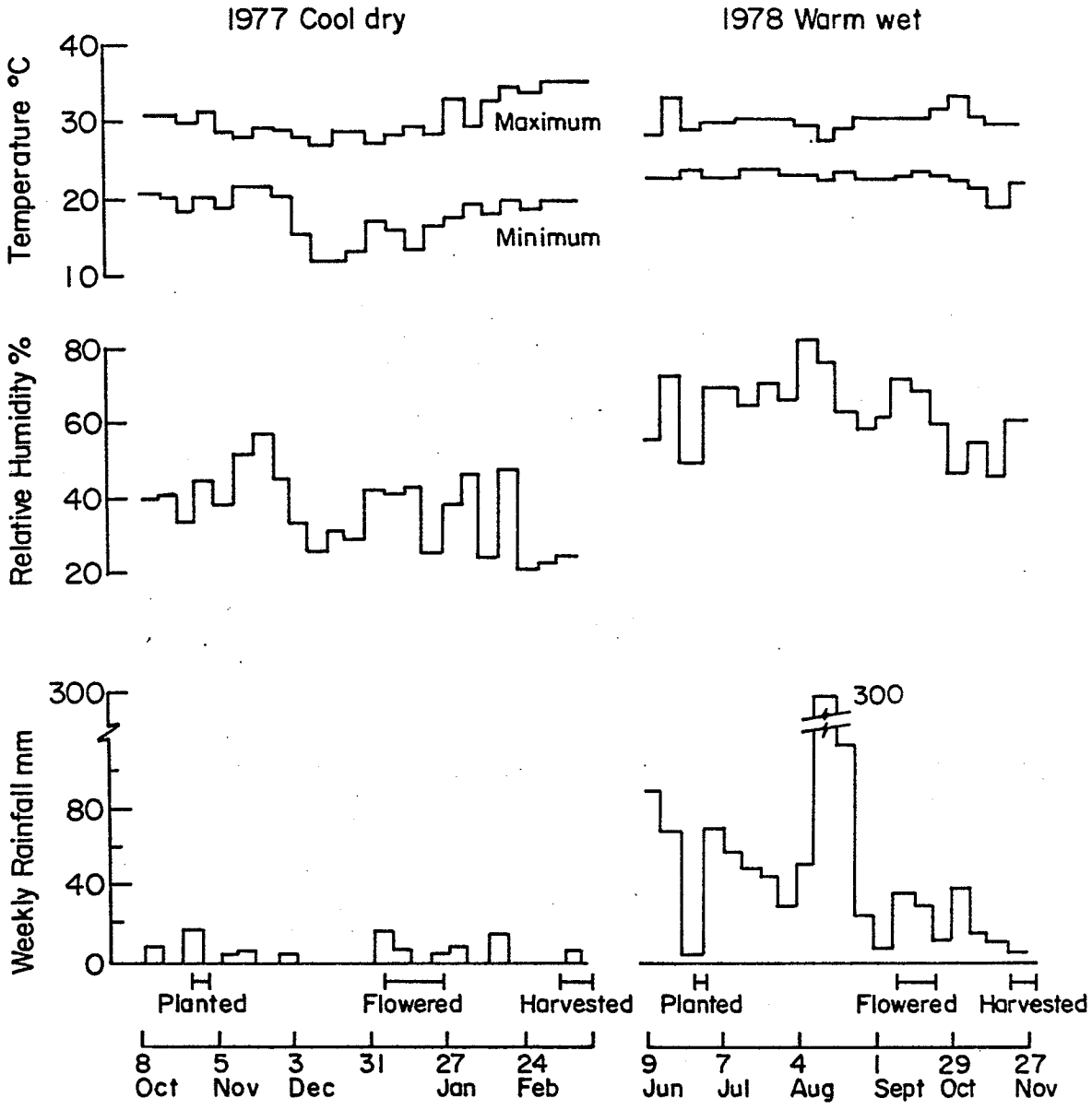

Figure 3.1. Weekly temperatures, relative humidity at 2 pm, and weekly rainfall during 2 seasons at ICRISAT center.

during most of the season. Excess rain at six weeks after planting caused water-logging on red soil sites. Among the off station locations grown during 1978 (Figure 3.2), Hissar had relatively uniform maximum temperatures and both minimum temperatures and relative humidity readings rapidly declining from one month after planting. Excess rainfall before planting caused flooding and delayed planting at this site. The weather at Parbhani resembled the warm wet season at ICRISAT Centre, except that minimum-maximum temperatures were more extreme, and rainfall was adequate but not in excess during growth. During the 1978 hot dry season at Bhavanisagar, temperatures were high during flowering and grain filling, and light rains occurred around planting and flowering.

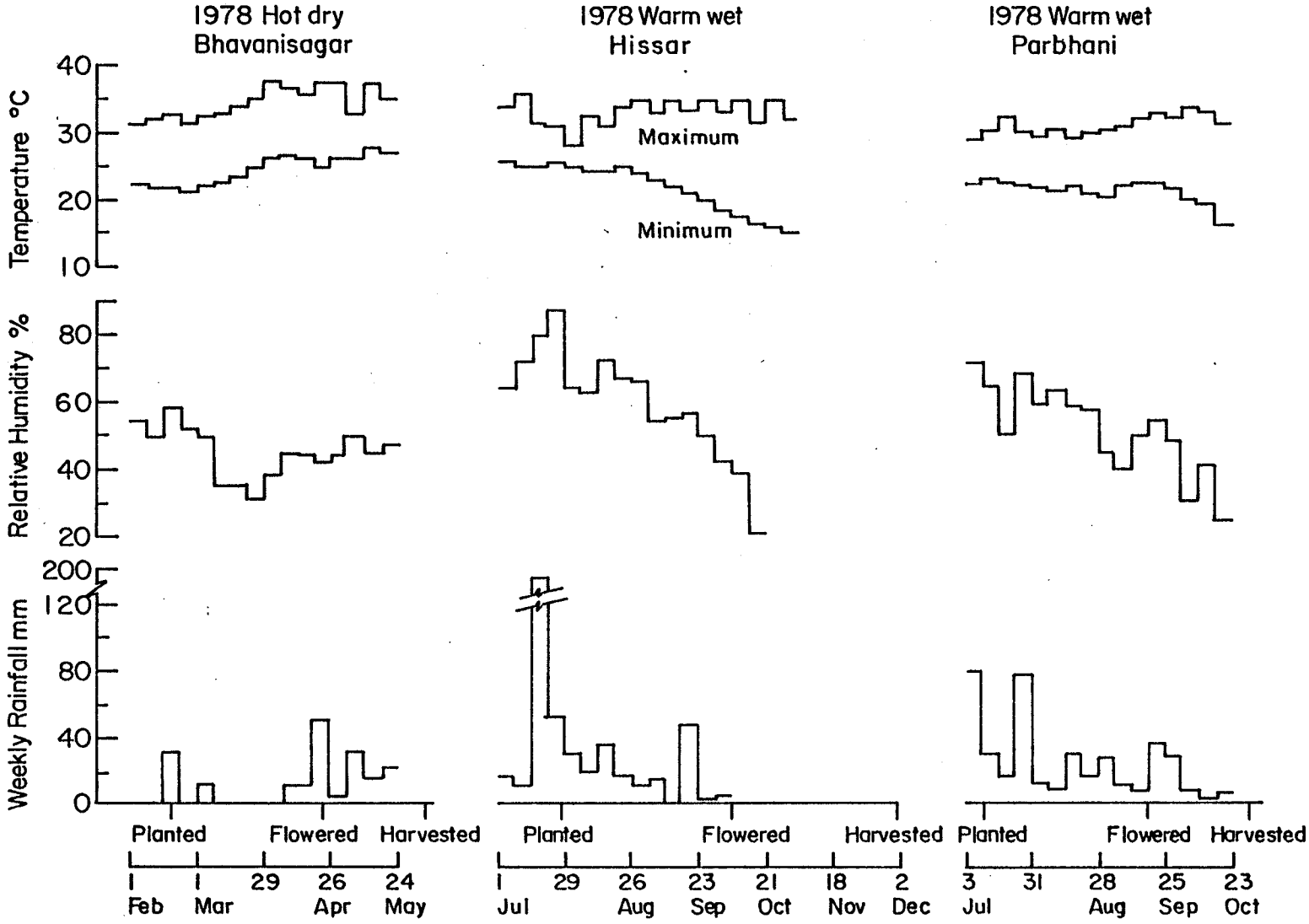

Figure 3.2. Weekly temperatures, relative humidity and rainfall at three off-station locations, during growth of trials.

RESULTS AND DISCUSSION

Equality of Error Variances

Error variances and trial means are presented in Table A.3. Variances were found to be heterogeneous for all plant variables. The existence of any relationship between site variances (σ_b^2) and site means was tested, in order to find a suitable transformation. No correlation was found between means and variances for any variable except for grain yield. However, since the maximum mean was less than three times the minimum mean, a transformation for grain yield would not be expected to be effective. Therefore, sites were combined on non-transformed data. It would appear that the requirement of homogeneity of error variances is not likely to be met when sites are combined from a wide diversity of environments.

Effect of Combining Both High and Normal Lysine Genotypes

The magniture of variances associated with environmental components were compared in high lysine, normal lysine, and combined groups of genotypes from the irrigation experiment (Table 3.2). The magnitude of variances in the normal lysine and high lysine subsets are different, but the combined genotype set had environmental variances much closer to the normal subset than to the high lysine subset. This is not surprising since 18 out of 22 genotypes were normal lysine. It does demonstrate that the environmental response of the combined genotypes set can be regarded as essentially that of a normal genotype set. The environmental response in the two-season genotype set, in which an equally high proportion

TABLE 3.2 The effect of combining high lysine and normal lysine genotypes on the size of variances associated with environmental components. Variances calculated from expected mean squares, using values of estimated lysine from the irrigation experiment.

Component	Variances		
	High lysine subset (4 genotypes)	Normal lysine subset (18 genotypes)	Combined set (22 genotypes)
(I) Irrigation	3.88	9.51	8.40
(S) Soil type	5.18	17.60	15.60
I x S	9.00	2.79	2.96
(N) Nitrogen	NS	6.05	4.00
S x N	NS	.32	.12
I x S x N	NS	NS	NS

15 of the 18 genotypes) were normal lysine, can also be considered to be that of a normal lysine genotype. Therefore, it appeared that the genotype sets in both the two-season and wet-dry experiments had normal lysine responses.

Relative Size of G x E Variances

The sizes of the genotypic variance (σ^2_g), environmental variance (σ^2_e) and genotype by environment variance (σ^2_{ge}) were compared for protein percent, estimated lysine, and grain yield (Table 3.3). Both σ^2_g and σ^2_e for protein and lysine appeared approximately equal in magnitude while σ^2_e was larger than σ^2_g for grain yield. The interaction variance (σ^2_{ge}) was smaller than σ^2_g or σ^2_e, but highly significant for all three characters. This interaction variance was approximately

TABLE 3.3. Expected and observed mean squares and variances for genotype (G), environment (E), and G x E components in the 2-season experiment.

Component	df	Expected mean square	Protein (%)		Est. lysine (% in protein)		Grain yield (kg/ha x 10^3)	
			MS	σ^2	MS	σ^2	MS	σ^2
(E) Environment	15	$\sigma_a^2 + \sigma_{ge}^2 + 18\sigma_e^2$	115.800	6.20	1.1530	.0604	45,030	2,425
Error a	24	σ_a^2	3.226		.0370		703	
(G) Genotype	17	$\sigma_b^2 + 16\sigma_g^2$	100.200	6.22	1.5670	.0970	24,840	1,527
G x E	255	$\sigma_b^2 + \sigma_{ge}^2$	1.821	1.28	.0422	.0273	1,080	679
Error b	816	σ_b^2	.537		.0149		402	
Rep/Site	24							
Total:	1151							

1/5 as large as σ^2_g for protein, 1/4 as large as σ^2_g for estimated lysine, and 1/3 as large as σ^2_g for grain yield. In this data set, σ^2_{ge} was a relatively small part of the total variance, but its relative size varied from one plant character to another. For protein percent, σ^2_{ge} was least important, more important for estimated lysine, and most important for grain yield.

Response to Environmental Factors

The significance of each of the environmental factors and interactions in the two-season experiment, was found from the appropriate F tests, and variances of the components were calculated from the expected mean squares formulae (Table A.3). These variances are tabulated in Table 3.4. Means for each environment are found in Table 3.5 for the ICRISAT sites, and in Table 3.6 for the off-station sites. Histograms of means for some important effects and interactions for seven plant characters are presented in Figures 3.3 to 3.9.

Percent Protein. (Figure 3.3). There was no significant difference in mean protein levels between either the two seasons, or the two management levels. A season by soil interaction occurred, with higher protein produced in red soil compared to black soil sites during the warm wet season, but with no significant differences in soil type means during the cool dry season. Protein levels in the black soil sites without nitrogen top dressing were quite low. Top-dressed nitrogen fertilizer was the most important single factor in increasing protein, and produced a larger increase on the black soil than on red soil sites, as the protein levels were quite high in the red soil plots even without nitrogen top dressing. The high protein level in the LN treatment was due

TABLE 3.4. Variances associated with the components in the two-season experiment, for protein percent (Prot.), estimated lysine percent in protein (Est. Lys.), grain yield in Ka/ha (Yield), 100 kernel wt(g) (KW), number of days to half flower (DF), and endosperm vitreousness (Vit.). NS signifies those components for which the F test was not significant at the 0.01 probability level.

Component	Variances					
	Prot.	Est. Lys.	Yield	KW	DF	Vit.
Season (Y)	NS	NS	NS	.359	NS	.599
Management (M)	NS	.0277	1059	.088	212.0	.063
Y x M	NS	NS	181	NS	107.5	.100
Soil (S)	1.64	.0328	NS	.068	NS	NS
Y x S	2.42	NS	2834	.022	88.2	.140
M x S	.21	.0055	787	NS	41.8	NS
Y x M x S	NS	NS	261	NS	33.0	.539
Nitrogen (N)	5.61	.0458	1008	NS	10.5	.112
Y x N	.99	NS	459	NS	4.5	NS
M x N	NS	NS	107	.016	NS	NS
Y x F x N	NS	NS	306	.032	5.0	NS
S x N	4.00	.0468	126	.011	5.5	.068
Y x S x N	1.31	.0272	311	.026	21.0	NS
M x S x N	NS	NS	127	NS	NS	NS
Y x M x S x N	NS	NS	NS	NS	NS	NS
Genotype (G)	99.90	.0961	1529	.379	49.4	1.730
G x Y	1.06	.0180	443	.135	8.1	.493
G x M	.71	NS	98	NS	NS	.126
G x Y x M	.31	NS	281	NS	NS	NS
G x S	.20	.0080	75	.029	NS	.096
G x Y x S	.53	NS	166	.174	NS	NS
G x M x S	NS	NS	325	.012	NS	NS
G x Y x M x S	0.82	NS	NS	.057	NS	.315
G x N	0.15	.0081	70	.012	NS	.047
G x Y x N	NS	.0097	NS	.026	NS	NS
G x M x N	NS	NS	NS	NS	NS	.016
G x Y x M x N	NS	NS	NS	NS	NS	NS
G x S x N	NS	NS	NS	NS	NS	.076
G x Y x S x N	NS	NS	NS	NS	NS	NS
G x M x S x N	NS	NS	NS	NS	NS	NS
G x Y x M x S x N	NS	NS	NS	NS	NS	NS

TABLE 3.5. Means and standard errors ($S\bar{x}$) of plant characters from the two-season experiment at ICRISAT.

Season	1977 Cool-dry				1978 Warm-wet				$S\bar{x}$
Management	High		Low		High		Low		
Soil	Black	Red	Black	Red	Black	Red	Black	Red	
Nitrogen				Protein %					
High (HN)	11.73	11.22	11.75	11.73	10.70	10.68	10.78	10.85	
Low (LN)	9.44	9.76	8.47	9.58	8.06	10.90	7.72	11.16	.122
				Estimated lysine (% of protein)					
HN	2.13	1.95	2.19	2.17	2.06	2.10	2.15	2.21	
LN	2.31	2.09	2.46	2.29	2.38	2.06	2.42	2.17	.011
				Estimated lysine (% of sample)					
HN	0.25	0.22	0.26	0.25	0.22	0.22	0.22	0.24	
LN	0.28	0.20	0.21	0.22	0.19	0.22	0.19	0.24	.011
				Protein (kg per ha)					
HN	317	427	239	285	370	392	346	221	
LN	236	329	136	215	206	186	222	125	20.0
				Grain Yield (kg per ha)					
HN	2795	3888	2242	2586	3524	3757	3437	2081	
LN	2561	3446	1700	2326	2624	1789	3006	1155	76.0

TABLE 3.5. Continued

Season	1977 Cool-dry				1978 Warm-wet				$S\bar{x}$
Management	High		Low		High		Low		
Soil	Black	Red	Black	Red	Black	Red	Black	Red	
				Kernels per head					
HN	830	1141	673	784	1468	1418	1525	930	44.3
LN	764	1078	550	721	1230	662	1311	466	
				100 kernel weight (g)					
HN	2.54	2.85	2.36	2.59	2.26	2.29	1.97	1.92	.025
LN	2.53	2.81	2.32	2.58	2.07	2.35	2.05	2.22	
				Heads per hectare (x 10^3)					
HN	135	121	145	131	108	115	115	118	.968
LN	136	136	117	137	129	117	112	110	
				Vitreousness score					
HN	3.75	3.45	2.97	3.38	2.70	2.78	2.95	2.57	.054
LN	3.08	3.35	2.55	3.30	2.53	2.71	2.69	2.40	
				Days to flower					
HN	78	69	89	75	70	69	72	77	.659
LN	78	70	89	76	70	76	73	84	

TABLE 3.6. Means and standard errors ($S\bar{x}$) of plant characters at 3 off-station sites.

Plant Character	Hissar 1978 Warm Wet		Parbhani 1978 Warm Wet		Bhavanisagar 1978 Summer		$S\bar{x}$
	HN	LN	HN	LN	HN	LN	
Protein %	11.38	11.97	9.76	8.47	11.25	8.80	.089
Est. Lys. (% in protein)	2.23	2.24	2.28	2.41	2.12	2.38	.014
Est. Lys. (% of sample)	0.25	0.26	0.22	0.20	0.24	0.21	.012
Prot/ha (kg)	562	543	392	314	480	237	16.9
Grain Yield (kg/ha)	4985	4802	4110	3786	4916	2740	64.5
Kernels/head	2074	2047	1740	1687	1090	844	33.9
100 kernel wt (g)	1.93	1.89	2.14	2.55	2.55	2.36	.021
Heads/ha (x 10^3)	125	125	108	103	169	138	.730
Vitreousness score	2.96	2.78	2.70	2.54	2.30	1.85	.056

HN = 150 kg N/ha applied as top dressing in addition to basal N.

LN = only Basal Nitrogen applied. (40, 60, 60 kg N/ha at Hissar, Parbhani, and Bhavanisagar, respectively.)

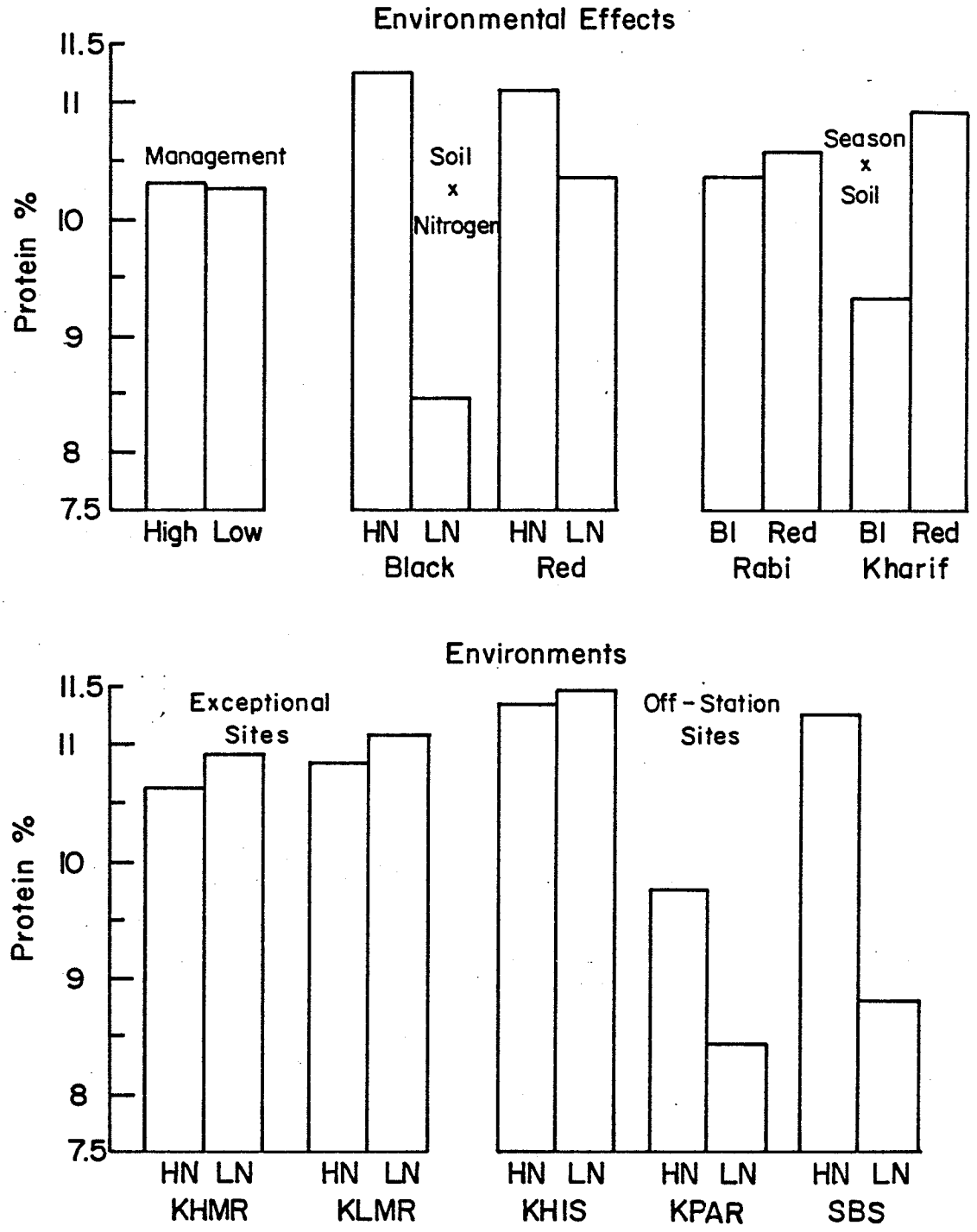

Figure 3.3. Means of environments and environmental effects for protein percent.
Black (or Bl) = Black vertisol Red = Red Alfisol
LN = basal N fertilizer only (20 to 60 Kg N/ha)
HN = 150 Kg/ha additional N in 2 topdress applications
Rabi = Cool dry season. Kharif (or K) = Warm wet season
HMR = High management red soil, LMR = Low management soil
His = Hissar, Par = Parbhani, SBs = Summer season Bhavanisagar

largely to the two red soil sites grown during the warm wet season, called KHMR and KLMR, which produced slightly, but not significantly, higher protein without nitrogen top dressing. These two sites are referred to as exceptional sites, and will be discussed later. Responses in protein at the three off-station sites could be compared with similar sites at ICRISAT. Both the fine sandy soil site at Hissar, and two red sandy soil sites at ICRISAT (KHMR and KLMR) produced high protein levels during the warm wet season, but no response due to the top-dressed nitrogen treatment. Low protein levels, especially in the LN treatment, were common to Parbhani and ICRISAT black soil sites during the warm wet season. Similar protein levels and a strong response to nitrogen top dressing were found in the red soil sites both during the hot dry season at Bhavanisagar, and during the cool dry season at ICRISAT.

Although the factor management had large effects on other plant characters (Table 3.5), there was no effect on protein percent due to different management levels or to interactions between management and other environmental factors. High management areas had received higher basal applications of both N and P than had the low management areas. It has been shown that such applications of fertilizer had no, or only slight effect on protein levels in sorghum (Warsi and Wright, 1973; Roy and Wright, 1973).

Estimated Lysine as Percent of Protein. (Figure 3.4). Examination of environmental effects on estimated lysine showed that conditions leading to high protein levels generally led to low estimated lysine levels and *vice versa*. Environmental factors which produced large variances for protein, generally produced large variances for estimated lysine as well

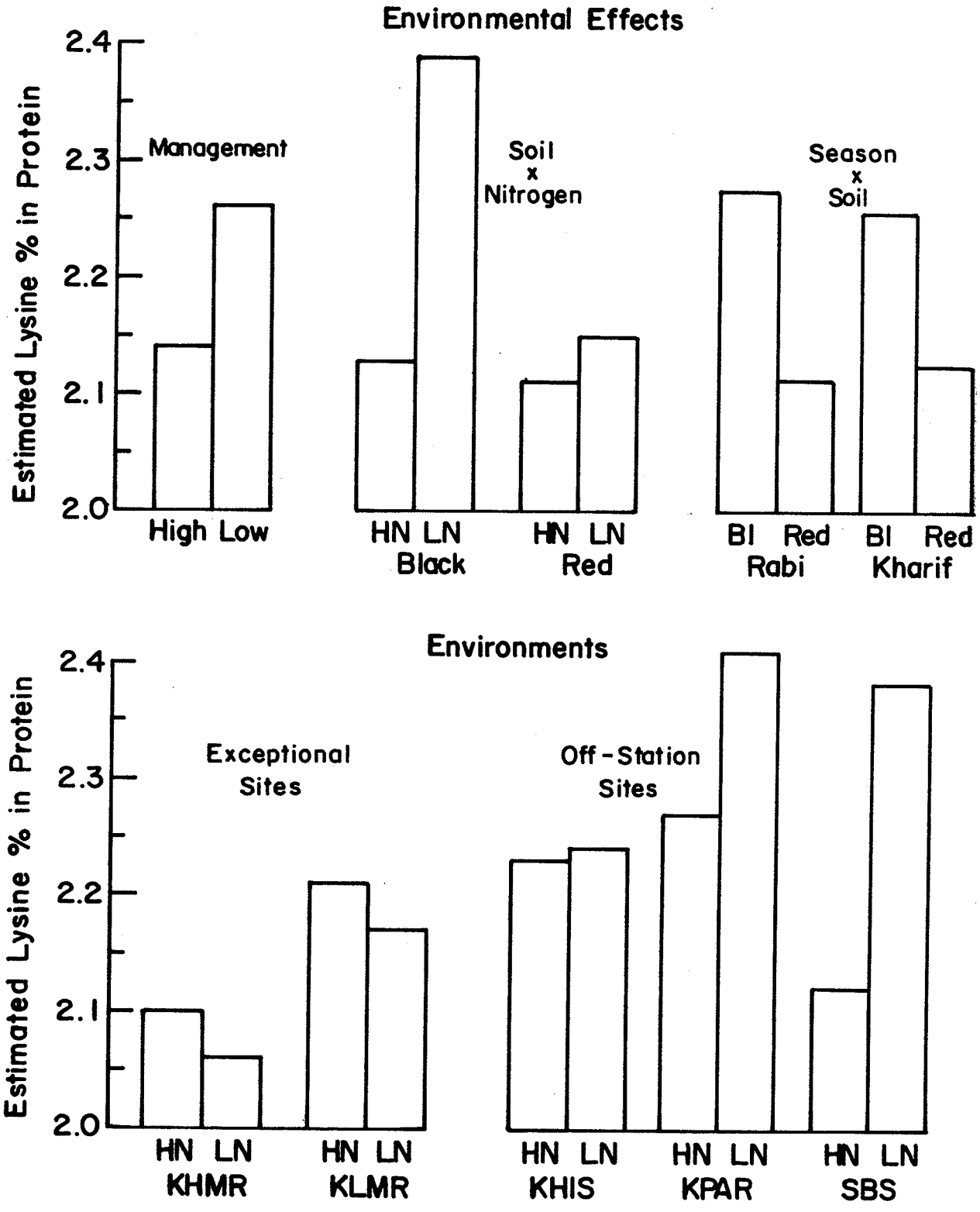

Figure 3.4. Means of environments and environmental effects for estimated lysine. Abbreviations explained in Figure 3.3.

(Table 3.4). This inverse estimated lysine-protein relationship existed in the large soil by nitrogen interaction and in individual environment means for protein percent and estimated lysine (Figures 3.3 and 3.4). The environments in the black soil without top-dressed nitrogen resulted in the lowest protein levels but the highest estimated lysine levels both at ICRISAT and Parbhani, with mean estimated lysine values of 2.4 percent of the protein. These values of estimated lysine were almost as high as the values found in the genetically high lysine sorghums (Manuscript 2, p. 58).

Two environmental components, *viz.* management and season by soil, were noted where size of the variance for estimated lysine was not related to that of protein percent (Table 3.4), which indicated that the inverse estimated lysine-protein relationship was not holding true for these effects. In both these cases, the level of estimated lysine appeared to be inversely related to the amount of protein per kernel, rather than to protein percent. The low management level produced higher estimated lysine, lighter kernels, but no difference in protein percent (Figures 3.4 and 3.6; Table 3.4). It would appear that at low management sites the smaller amount of protein per kernel, which is a product of kernel weight and protein percent, was inversely related to the higher estimated lysine value (Figures 3.4 and 3.5). In the second case, there was no difference in protein percent due to soil type during the cool dry (Rabi) season, but black soil produced lower protein percent than red soil sites during the warm wet (Kharif) season (Figure 3.3). Estimated lysine was higher in the black soil sites during both seasons (Table 3.5). This resulted in a fairly large Y x S variance

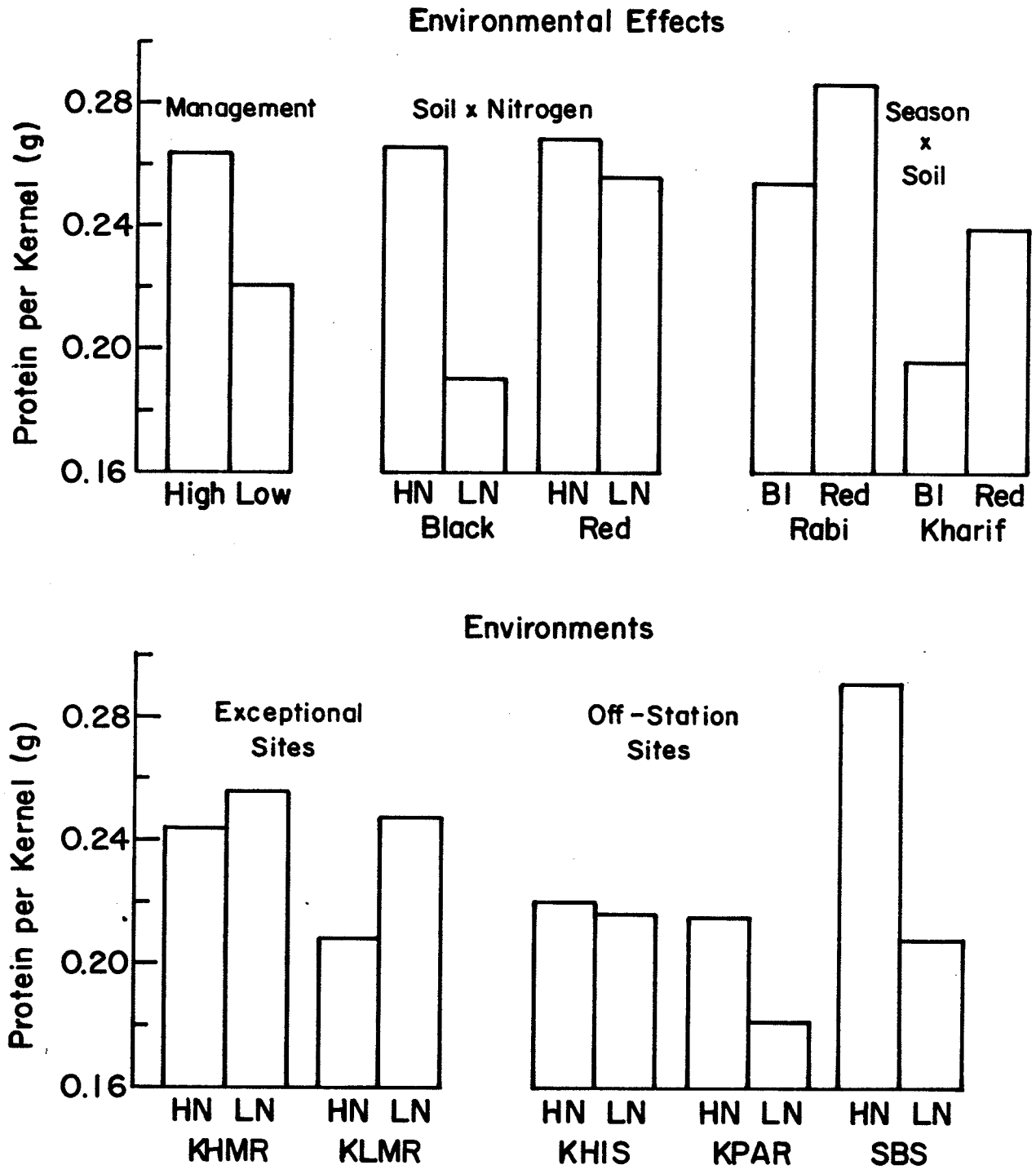

Figure 3.5. Means of environments and environmental effects for protein per kernel. Abbreviations explained in Figure 3.3.

for protein percent, but no significant Y x S interaction for estimated lysine (Table 3.4). The smaller kernel weight in the black soil compared with red soil sites during the cool dry (Rabi) season (Figure 3.6), resulted in less protein per kernel (Figure 3.5). It appeared that estimated lysine was inversely related to protein per kernel in the Y x S interaction, as well as in other environments and environmental effects.

Kernel Weight (Figure 3.6). Season was the most important factor for this variable, as heavier kernels were produced in the cool dry than in the warm wet season. The heavier kernel weight produced by the high management sites, and from red soil sites during the cool dry (Rabi) season, has already been mentioned in relation to estimated lysine (p. 94). Top-dressed nitrogen did not produce any change in kernel weight in the two-season experiment, but produced slightly, but not significantly heavier kernels in all three off-station sites. In both the exceptional sites, top dressed nitrogen resulted in lighter kernel weight. Application of nitrogen fertilizer has previously been found to have either no effect, or to reduce kernel weight in sorghum (Roy and Wright, 1973).

Grain Yield (Figure 3.7). The large environmental variances associated with the M and N factors (Table 3.4) were a result of higher grain yields in high management environments and in environments where top dressed nitrogen was applied (Table 3.5). Neither season nor soil type had a significant effect on grain yield, but the large season by soil interaction indicated that the red soil sites were producing higher yields during the cool dry season, but that the black soil sites yielded most in the warm-wet season.

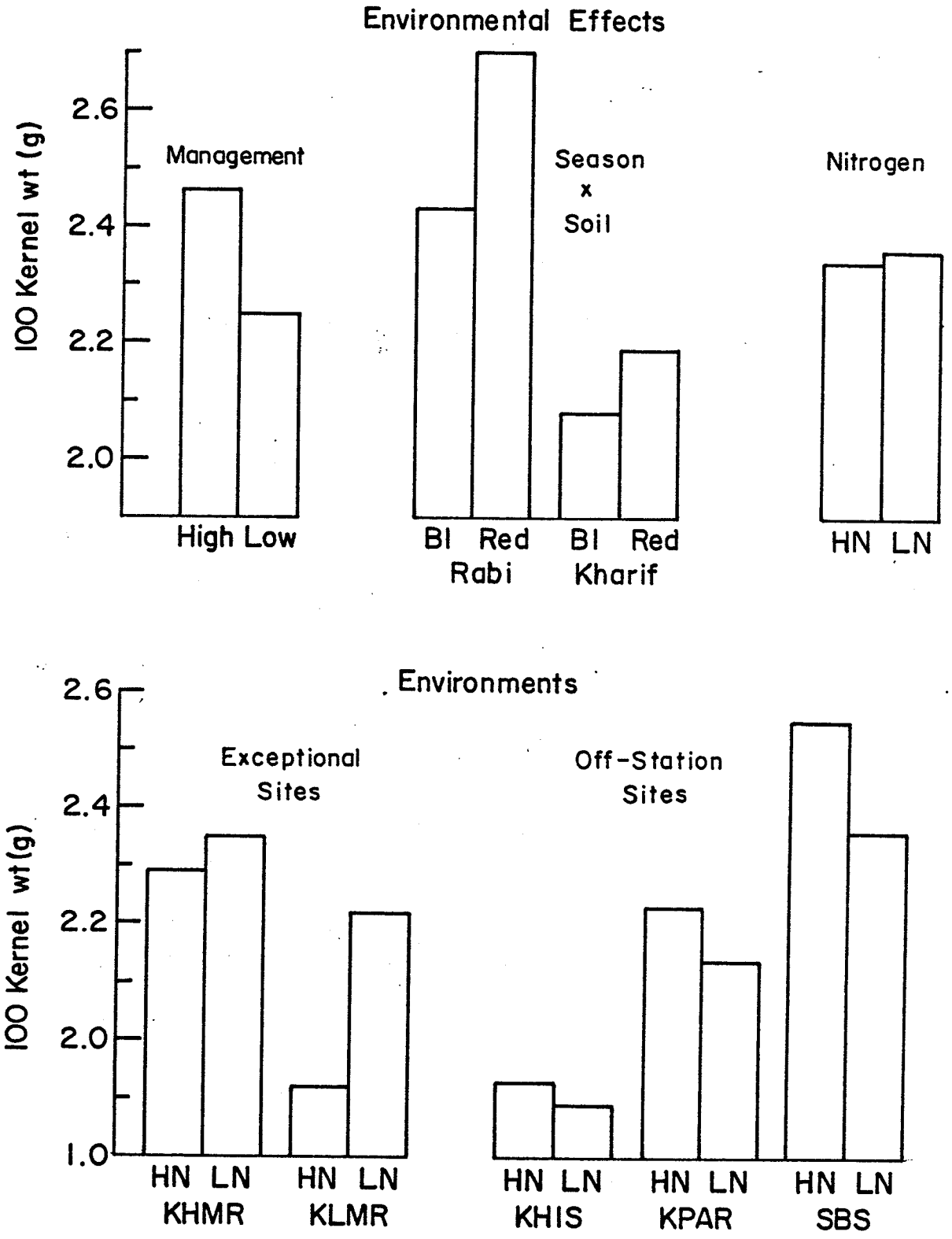

Figure 3.6. Means of environments and environmental effects for 100 kernel weight. Abbreviations explained in Figure 3.3.

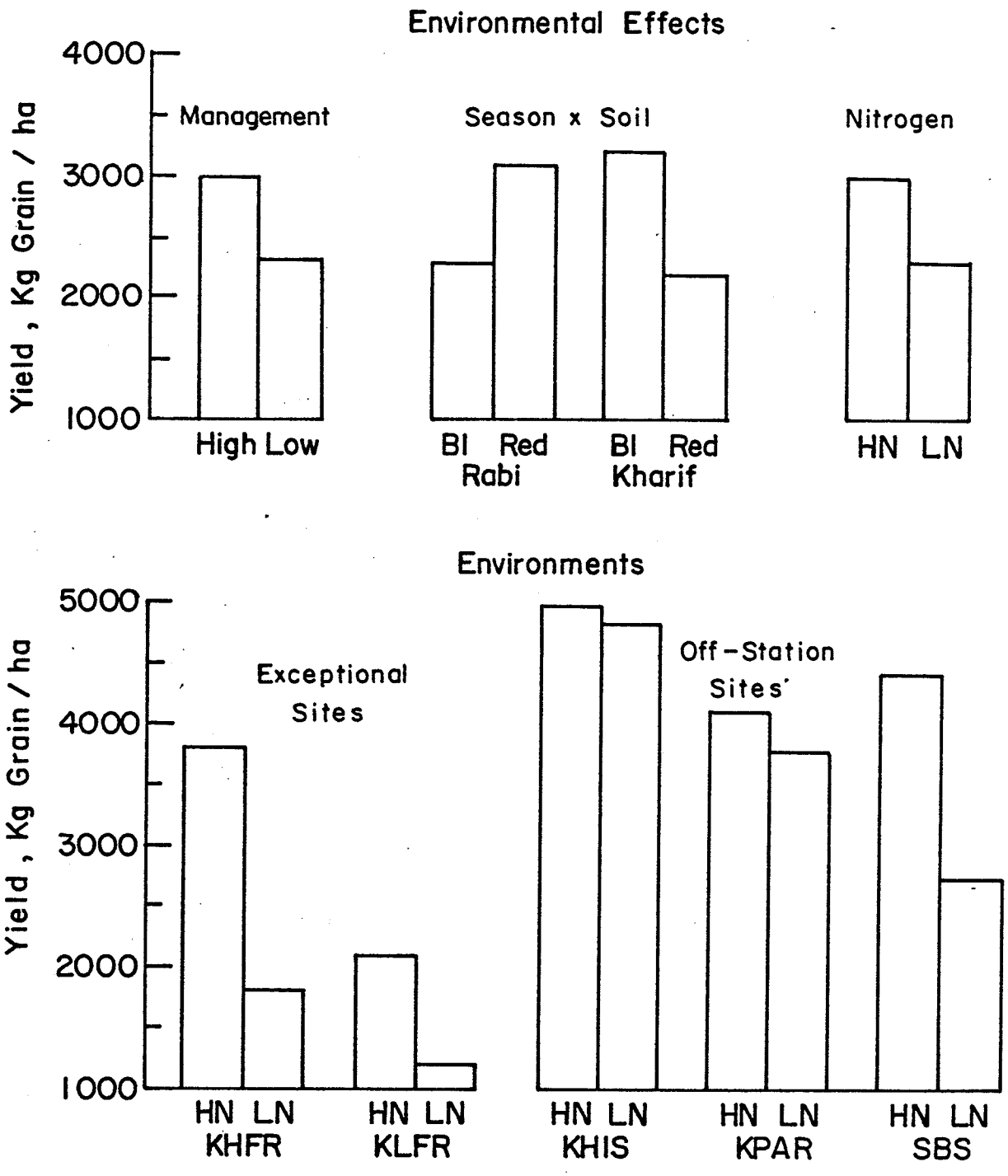

Figure 3.7. Effects of environmental factors and environments on grain yield. Abbreviations explained in Figure 3.3.

Most of the environmental effects for grain yield were not common with those for protein percent. For example, the high management sites produced higher yields, but no change in protein percent compared with low management sites; black soil sites during the cool dry season produced lower yields and no change in protein compared with red soil sites, but during the warm wet season,black soil sites produced higher yields and lower protein compared with red soil sites. Only the N factor was associated with a common yield-protein response. At any site where top dressed nitrogen was effective in increasing yield, it also significantly increased protein, except at the two exceptional sites, where top dressed nitrogen resulted in an increase in yield, but no significant change in protein percent. The yield-lysine relationship was the inverse of the yield-protein relationship so that there generally was no common environmental response between estimated lysine and grain yield. Again, the exception was the top dressed nitrogen treatment which produced lower estimated lysine, and higher yield across sites. Only at the two exceptional sites did the topdress nitrogen treatment result in higher yields but no significant change in estimated lysine.

Days to Flower (Figure 3.8). It appeared that flowering time was most affected by those factors which hampered growth. The delay in flowering both in low management and low nitrogen environments, indicated that lack of nitrogen, and possibly phosphorus, was slowing down the growth cycle in these environments. The delay in flowering in black soil sites in the cool dry season was due to a heavy attack of shootfly, killing primary culms. Heads at these sites were mainly from tillers, which were later in flowering. The delay in flowering in the red soil sites

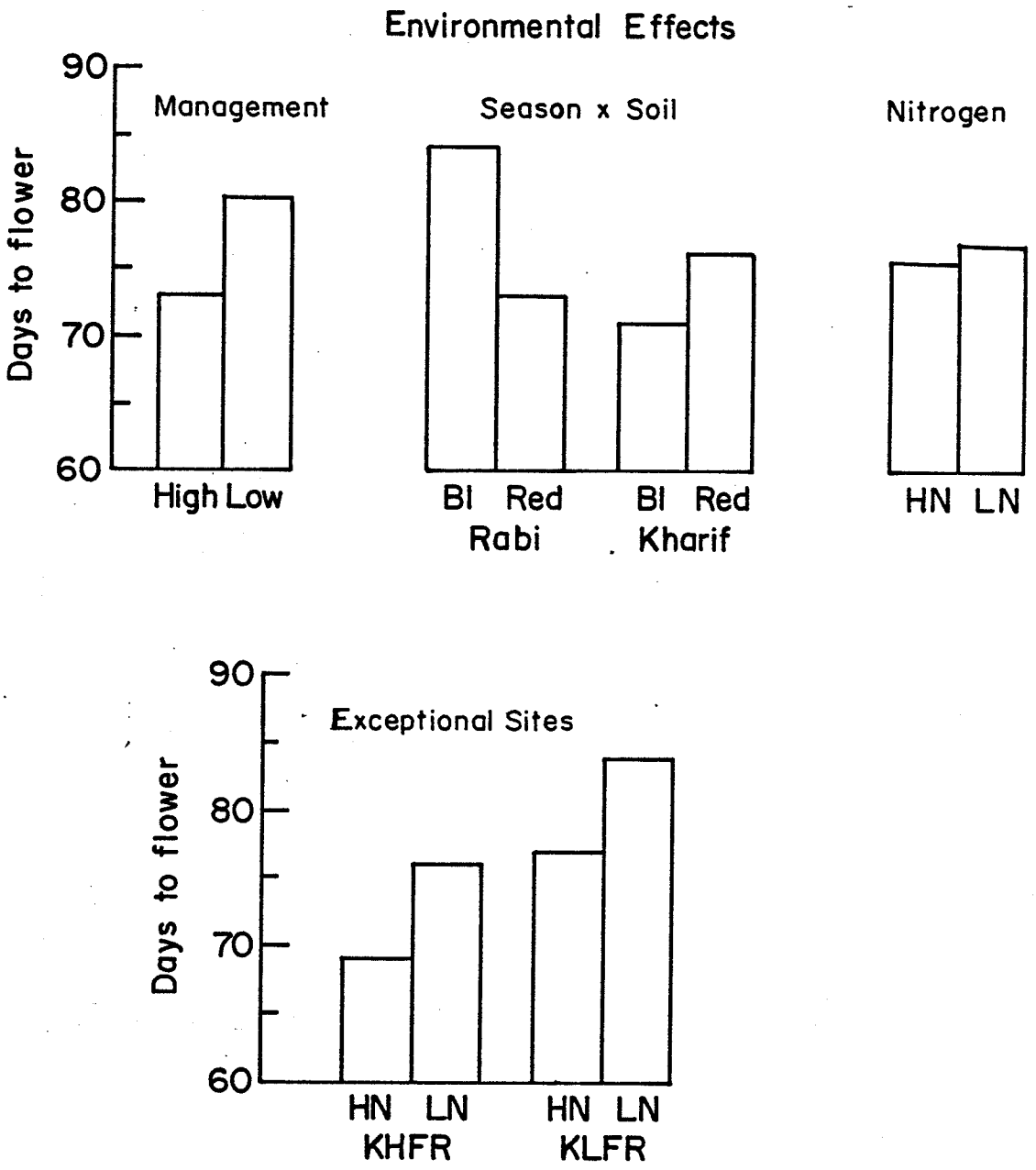

Figure 3.8. Effects of environments and environmental factors on days to flower. Abbreviations explained in Figure 3.3.

during the warm wet season was caused by waterlogging during and just after the panicle initiation stage.

Endosperm Vitreousness (Figure 3.9). The seasonal effect was the most important factor influencing this variable, with a higher vitreousness score, indicative of more vitreous endosperms, being produced in the cool dry than in the warm wet season. Top dressed nitrogen also resulted in a small increase in vitreousness. Hissar, in North India, produced grain with the highest vitreousness score, while Bhavanisagar, in South India produced the lowest vitreousness score.

Soil Analyses. Soil chemical analyses, taken at the end of grain filling, are presented in Appendix Table A.4. T-tests were computed for differences among the environment factors, and those that gave significant or unexpected results are presented in Tables 3.7 a-c. Surprisingly, there was no increase in available soil nitrogen due to top-dressing with nitrogen. In all environments, this test produced very low levels of available soil nitrogen. (Available nitrogen below 121 ppm was called low.) As expected, high fertility sites did have higher available phosphorus than did low fertility sites, but means of both levels were quite low (values below 12.4 ppm were called low.) Black soil sites had lower available nitrogen than did red soil sites, but had higher pH, and higher electrical conductivity, as well as higher available potassium and exchangeable sodium, indicating that the black soils tended to be more alkaline and saline than the red soils.

Even at high nitrogen rates, the available nitrogen in the soil was very low, which indicates that fertilizer nitrogen was disappearing quickly from these soils. Allison (1966) found that over half the

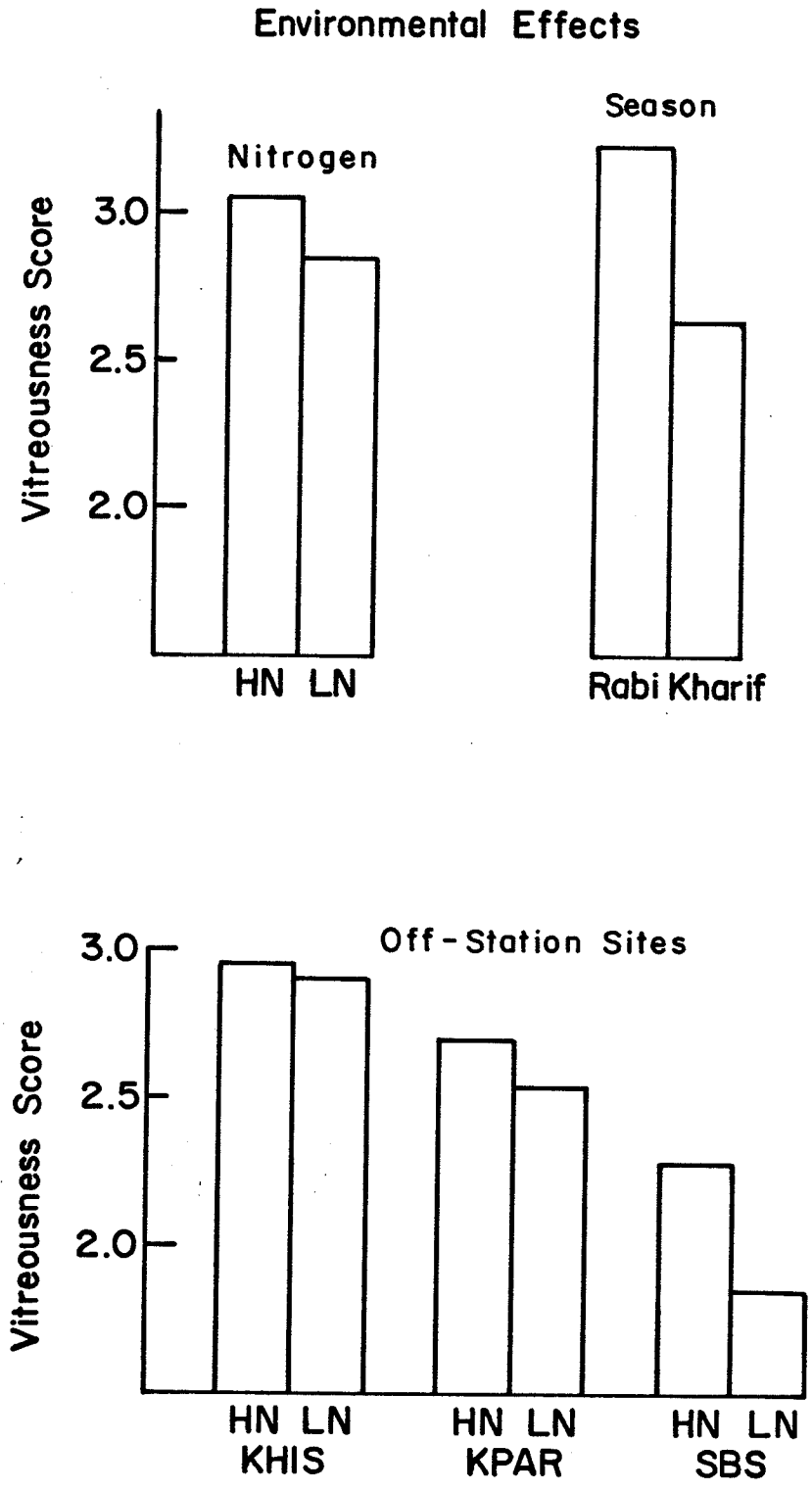

Figure 3.9. Effects of environments and environmental factors on vitreousness score (1 = floury endosperm, 9 = completely vitreous). Abbreviations explained in Figure 3.3.

TABLE 3.7 a-c. Values from soil analyses associated with environmental factors in the two-season experiment. Samples were taken at the end of the growing season.

TABLE 3.7a. Available soil nitrogen following high or low fertilizer nitrogen treatments.

	Fertilizer Nitrogen	Available Soil N (PPM)
	High	82
	Low	85
Difference: t-test		NS

TABLE 3.7b. Available N, available P, and exchangeable K values from high and low management areas.

	Management	Available N (ppm)	Available P (ppm)	Exch. K (ppm)
	High	80	4.1	150
	Low	86	2.5	193
Different:	t-test	NS	**	NS

TABLE 3.7c. Available N, available P, exchangeable K, exchangeagle Na, pH and electrical conductivity values from black and red soil sites.

Soil type	Avail. N (ppm)	Avail. P (ppm)	Exch. K (ppm)	Exch. Na (ppm)	pH	E.C. (m.mhos/cm)
Black (Vertisol)	63	3.9	236	519	8.3	.21
Red (Alfisol)	90	3.7	99	103	6.5	.10
Difference: t-test	**	NS	**	**	**	**

** Probability of difference being due to chance $P < 0.01$.

applied nitrogen fertilizer was generally lost from soils, mainly through leaching and gaseous losses from denitrification. High temperatures and periods of saturated moisture conditions could greatly increase nitrogen losses. However, he found that nitrogen could be remobilized as soils dried out.

In the two "exceptional" red soil sites during the 1978 warm wet season, waterlogging occurred from the 5th to the 8th week after planting, affecting the LN plots most severely, indicated by slower growth and pale green color of the plants. This stress occurred during and after panicle initiation, which is a time when seed numbers and hull size are determined (Murata, 1969). As the soil dried out during the time of flowering, it appeared that nitrogen became available, as even the plants on plots which had received no nitrogen top dressing became as dark green as the plants in HN plots. Since the kernel weight from the LN plots in these sites was heavier than in the HN plots, (2.28, compared with 2.10 g/100 kernels), it would appear that the waterlogging produced low yields by reducing kernel numbers. The low kernel numbers and apparent availability of nitrogen during grain filling may have resulted in more concentrated protein in the kernels and would explain the high protein levels produced in the LN environments at these two sites.

Effect of Moisture Stress

Some responses to moisture stress on a red and a black soil site for four plant characters are presented in Figure 3.10. Means and standard errors for all plant characters measured are reported in Table 3.8.

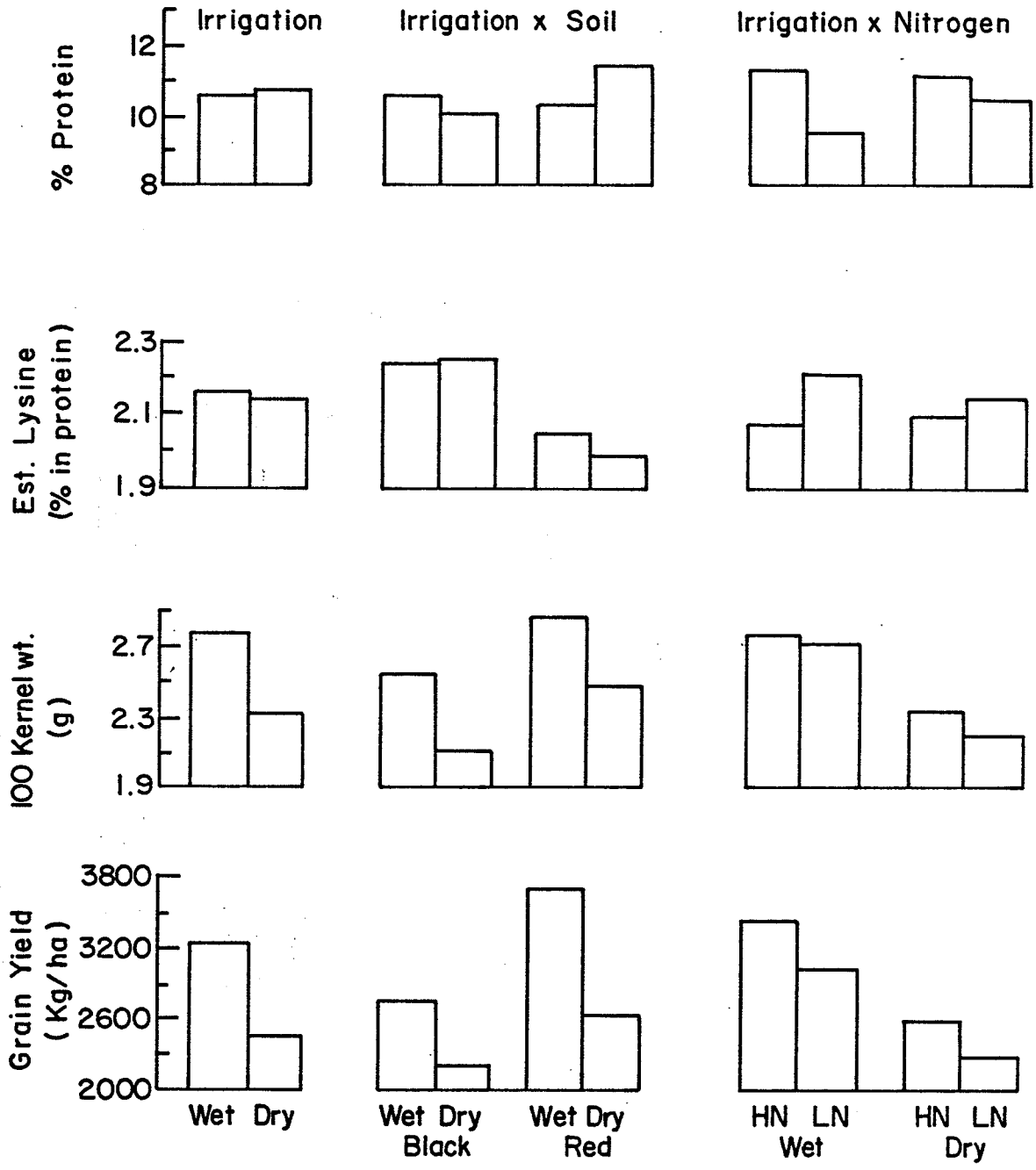

Figure 3.10. Effect of full irrigation (wet), or restricted irrigation (dry), and nitrogen top dressing (HN) or only basal nitrogen fertilizer (LN) in a Black and Red soil during the 1977 cool dry season at ICRISAT.

TABLE 3.8. Environment means and standard errors ($S\bar{x}$) of plant characters in the irrigation experiment during the 1977 cool dry season at ICRISAT.

Plant Variable	Environments*								$S\bar{x}$
	BW		BD		RW		RD		
	HN	LN	HN	LN	HN	LN	HN	LN	
Protein (%)	11.71	9.50	10.10	10.03	11.15	9.69	12.10	10.80	.045
Est. lysine (% in protein)	2.17	2.35	2.27	2.28	1.99	2.12	1.95	2.04	.006
Est. lysine (% of sample)	0.25	0.22	0.23	0.22	0.22	0.20	0.24	0.22	.004
Grain yield (Kg/ha)	2867	2552	2414	2078	3942	3523	2732	2492	38.1
100 Kernel wt (g)	2.61	2.58	2.17	2.11	2.91	2.84	2.52	2.44	.006
Kernels per head	851	758	796	751	1161	1094	915	883	19.6
Heads/ha (x 10^3)	132	133	145	134	121	118	124	119	1.06
Plant height (cm)	122	120	110	104	140	137	124	126	.934
Days to flower	79	78	75	76	70	71	70	70	.234
Vitreousness score	3.6	2.9	3.4	3.2	3.2	3.1	3.1	3.2	.045
Protein/ha (Kg)	326	234	240	204	431	334	322	264	14.1

*Environment abbreviations: B = Black Soil, R = Red soil
W = Fully irrigated, D = Restricted irrigation
HN = 198 Kg N/ha in 3 applications
LN = 48 Kg N/ha applied at planting

Protein percent was increased by moisture stress in the red soil site but was reduced by moisture stress in the black soil site. This interaction in reverse was also true for estimated lysine. Kernel weight, yield and plant height were significantly reduced by moisture stress in both red and black soils. The different response to stress in the red and black soils might be explained by the different nature of the stress in the two soils. In the black soils, a prolonged, but mild stress occurred throughout the growing season. This type of stress reduced the size of the grain and hence the yield, on both red and black soil sites.

There was no change in protein level due to topdress N at the stressed black soil site (Table 3.8) presumably because the moisture in the soil was not sufficient to move enough top dressed N down to the roots to have any effect on seed protein level. The higher protein level with N top dressing at the irrigated black soil site, resulted in higher mean protein at the irrigated, compared with the stressed black soil environments. In the red soil environments, moisture stress did not occur early in the season, but became progressively more acute during seed filling. This stress caused a reduction in kernel size and in yield, but appeared to have less effect on translocation of nitrogen compounds into the grain, and resulted in a higher concentration of protein in the smaller kernels.

CONCLUSIONS

In these predominantly normal lysine genotype sets, a negative protein to estimated lysine relationship was apparent for most, but not all environments or environmental factors in these experiments. Estimated lysine levels appeared to be more closely and inversely related to protein per kernel than to protein percent, especially for those environmental factors where the protein percent-estimated lysine relationship was not apparent.

It appeared that plants at many of the environments tested were stressed for nitrogen, and that nitrogen availability in the soil fluctuated during the growing season. This was indicated by the increases in both protein percent and grain yield produced by the nitrogen topdress treatment; the non-availability of that nitrogen in moisture stressed black soils; the occurrence of waterlogging at some sites causing nutrients including nitrogen, to be unavailable; and the failure to find any difference in available soil nitrogen due to the application of nitrogen top dressing at the end of the growing season.

The black soil sites without N top dressing produced grain with protein as low as 7.7 percent, with estimated lysine of 2.41 percent of the protein. This level of estimated lysine was close to the mean of 2.54 found in the P-721 high lysine mutant (Manuscript 2, page 58). Studies which monitored protein levels of grain crops in India (von Oppen, 1979; ICRISAT, 1977) indicated that sorghum from farmers' fields averaged only 8.2 percent protein. Although lysine values were not determined, the farmers' sorghums with low protein values might be

expected to possess high levels of lysine (as percent of protein).

Plants which were subjected to continuous but mild moisture stress in the black soil environments, responded differently from those subjected to a more acute stress during flag leaf and grain filling stages in the red soil environments. Kernel weight as well as grain yield were reduced by both types of stress. However, protein percent decreased with the stress in black soil environments, and increased with stress in the red soil environments. The continuous moisture stress in the black soil appeared to make the fertilizer nitrogen unavailable to the plant roots, resulting in a lower protein level. The acute stress in the red soil site during grain filling reduced kernel weight, and apparently resulted in a concentration of the protein in the smaller kernels.

There was no significant change in mean values of protein or grain yield between the warm wet season and the cool dry season at the sites tested. It may be that other factors were more important than the difference in day-night temperatures and in relative humidity, which distinguish these seasons (Figure 3-1 and 3-2). For example, an important season by soil interaction occurred because high rainfall during the warm wet season caused waterlogging only in red soil sites and resulted in higher protein values and lower yields than in the black soil sites.

Although it appeared possible to account for a large amount of the environmental response at these sites where climatic, soil, and imposed environmental factors were measured, much of the variation was contained in interactions, requiring specific knowledge of conditions

at the sites during the growing season in order to interpret the responses. Interrelationships between plant variables were not consistent, but changed from one environmental factor to another. The specific nature of responses at these environments argues for the testing of genotypes in environments as nearly identical as possible to those in which the future variety is expected to be grown.

Genotype by environmental variances in the two-season experiment which used 18 genotypes (three of which were high lysine) in 16 environments, were found to be significant for protein percent, estimated lysine, and grain yield. Although σ^2_{ge} was considerably smaller than σ^2_g, it was proportionately greater for grain yield and estimated lysine than for protein percent. The different environmental responses in genotypes, indicated by σ^2_{ge}, are investigated in Manuscript 4.

MANUSCRIPT 4

A COMPARISON OF THE ENVIRONMENTAL RESPONSES OF SOME HIGH AND NORMAL LYSINE SORGHUM GENOTYPES IN THE SEMI ARID TROPICS

INTRODUCTION

Most of the identification and description of the high lysine sorghum mutants, as well as of high lysine mutants in other cereals have been carried out on plants grown under favorable agronomic conditions with adequate nutrients and moisture during the growing season. The existence of a genotype by environment interaction for estimated lysine (Manuscript 3), however, indicated that the relative lysine levels or the ranking of the lines changed in different environments.

A number of experiments with the high lysine barley mutants, Hiproly and Riso 1508, and Opaque-2 maize (Anderson and Koie, 1975; Zink and Wilberg, 1976) have shown that increments of fertilizer nitrogen resulted in a decrease in lysine (as percent of protein) in normal lysine lines, but caused less or no reduction in lysine in the high lysine lines. Seed protein percentage increased both in normal and in high lysine lines with higher rates of nitrogen. When the proteins in the lines from the different nitrogen treatments were fractionated, it was found that the difference in lysine levels was related mainly to the amounts of the prolamine fraction, or storage protein, which characteristically possesses a very low lysine content (Nelson, 1978). In the normal lysine lines, the proportion of prolamine protein increased with increments of nitrogen fertilizer, but in the high lysine lines, in which the prolamine fraction was genetically suppressed, increments of nitrogen were found to increase the proportion of prolamine only slightly, or not at all. It was concluded that the production of high quality protein in these lines could be maximized when grown at high levels of fertilizer nitrogen.

However, in large areas of the semi-arid tropics, heavy applications of fertilizer nitrogen are not feasible. On the adequately fertilized research fields at ICRISAT, protein in sorghum averaged 12 percent (ICRISAT, 1977), while, sorghum arriving at grain markets from farmers' fields over a large part of semi-arid tropical India averaged only 8.2 percent (von Oppen, 1979). It would appear that high lysine sorghums would have potential use in these areas which are producing low protein sorghum, and any interaction in the performance of high lysine and normal sorghums between research and farmers' conditions needs to be examined closely.

This manuscript reports an investigation of the lysine levels, as well as protein levels and grain yield, in both normal lysine and high lysine sorghum lines over a range of environments in the semi-arid tropics, and a comparison of differences in protein solubility fractions of endosperms from a high lysine and a normal lysine line grown in high and low nitrogen environments.

MATERIALS AND METHODS

Two sets of genotypes were compared over environments. The first set, composed of entries in trials known as stability study 1, was tested over sites in four seasons. These sites are summarized in Table 4.1, and have been more thoroughly described in Manuscript 3. At each site, two soil nitrogen levels were imposed, with four replications at each level. Low nitrogen (LN) treatments received only a single application of 20 to 48 KgN/ha at planting. High nitrogen (HN) treatments received an additional 50 Kg at panicle initiation, and 100 KgN/ha when the crop had reached the boot leaf stage. These two treatments created two environments at each site.

Twenty-two lines were tested in the 1977 cool dry season. Four lines had high or moderately high levels of lysine, *viz*: the Ethiopian high lysine (*hl*) mutant, IS.11758; the Purdue high lysine mutant P-721; and two bulgy lines, derived from crosses with the *hl* source. The bulgy lines did not contain the *hl* gene but instead contained genes which appeared to condition both the bulgy appearance of the seed and the increased lysine concentration in the protein (Manuscript 2). Twelve of the lines were derived from the *hl* source, but had normal lysine levels and did not contain the *hl* gene. In addition, five adapted varieties and a popular Indian hybrid, CSH-1 were included. In the remaining three seasons, only 18 genotypes were tested. IS.11758, two farmers' varieties, and one normal lysine *hl*-derived line were removed, as they were adapted to the cool dry season only. The pedigrees of these lines are shown in Table A-1. Lines derived from crosses with *hl* parents are called *hl*-derived lines, even though it appeared unlikely

TABLE 4.1. Characteristics of trial sites in semi-arid tropical India on which stability investigations were conducted.

Site Abbrev.	Location and Latitude		Soil[a]	Management[b]	Rain/ Irrigation	Nitrogen[c] Levels
	1977 Cool dry season (Rabi)					
7R HMB W	ICRISAT Centre	18°N	Vertisol	High	Full irrigation	HN + LN
7R HMB D	"		Vertisol	High	Restricted	" "
7R HMR W	"		Alfisol	High	Full irrigation	" "
7R HMR D	"		Alfisol	High	Restricted	" "
7R LMB	"		Vertisol	Low	Full irrigation	" "
7R LMR	"		Alfisol	Low	Full irrigation	" "
	1978 Warm wet season (Kharif)					
8K HMB	ICRISAT Centre	18°N	Vertisol	High	Rainfed	HN + LN
8K HMR	"		Alfisol	High	"	" "
8K LMB	"		Vertisol	Low	"	" "
8K LMR	"		Alfisol	Low	"	" "
8K His	Hissar	29°N	Aridisol	-	"	" "
8K Par	Parbhani	19°N	Vertisol	-	"	" "
	1977 Warm wet season (Kharif)					
7K HMB	ICRISAT Centre	18°N	Vertisol	High	Rainfed	HN + LN
7K Bs	Bhavanisagar	11°N	Alfisol	-	Rainfed	HN only
7K His	Hissar	29°N	Aridisol	-	Rainfed	HN only
	1978 Hot dry season (Summer)					
8S Bs	Bhavanisagar	11°N	Alfisol	-	Full irrigation	HN + LN

[a] Soils: Vertisol, a black cracking clay; Alfisol, a red sandy loam; Aridisol, a fine sandy loam.

[b] Low management sites received 20 Kg N and 20 Kg P_2O_5/ha. High management sites received 38 to 48 Kg N and 60 Kg P_2O_5/ha at planting. Off-station sites received recommended rates, similar to high management sites.

[c] HN (High Nitrogen) environments received 150 Kg of top dressed N/ha. LN (Low Nitrogen) environments did not receive any nitrogen top dressing.

that these lines contained the *hl* gene.

The second set of lines consisted of 20 interrelated F_4 bulks. In creating this set, each of 5 *hl*-derived lines was crossed with P-721 and with another *hl*-derived line. Selection was carried out, during generation advancement, for agronomic appearance as rated by a field score, followed by either selection for high grain weight per head, or for high dye binding capacity/protein ratios. One F_4 bulked line for each of the 10 crosses and for each selection method was entered into stability study 2, along with P-721, CSH-1 and an adapted variety as checks. These 23 entries were evaluated over 12 environments during the 1978 warm wet season. The responses of four F_4 bulks from crosses with P-721, and selected for high lysine, were compared with the response of P-721 across environments.

The stability analysis of Eberhart and Russell (1966) was used in comparing the responses of individual high lysine and normal lysine genotypes over environments. This analysis produces three parameters: the mean of each genotype across all environments; a regression coefficient (b value) obtained from the regression of the genotype means onto an environmental index; and the deviations from regression (also expressed as a deviation mean square value) which measures the failure of linear regression to account for the genotype response. The environmental index is the mean of the genotype set at each environment. Since the genotype sets contained a predominance of normal lysine lines, the index was essentially the response of a set of normal lysine genotypes.

Agronomic and chemical evaluations were based on three metres in

the central row of three-row plots, and were described in Manuscript 3, (page 74). An analysis of variance was made on each plant character at each site. An assumption of the stability analysis is that error variances from different sites are homogeneous. According to Bartlett's test (Snedecor and Cochran, 1967) the error terms were not homogeneous (Table A,3). However, no transformation was found to be satisfactory, and the stability analysis was run on untransformed data.

The line IS.11758 was entered in only the short-day, cool, dry season environments in the first stability study, as this was the limit of its adaptation. Since the stability parameters for most characters for the lines tested in all 30 environments were very similar to those lines tested in only the cool dry season, only the cool dry season environments will be used to illustrate the responses of most of the characters tested.

A detailed investigation of the nature of the protein and lysine response in terms of kernel composition, was followed by an attempt to clarify the effect of environment on protein solubility fractions in relation to genotype differences in lysine levels. Single heads of P-721 and CSH-1 from high nitrogen (HN) and low nitrogen (LN) treatments at a single site (7RLFB) were chosen as having estimated lysine, protein percent and seed weights similar to their respective treatment means at this site. Endosperm , embryo, and pericarp were separated in 200 seeds per head. Endosperm lysine was analyzed by a Beckman 120c amino acid analyzer. The nitrogen in the endosperms was successively extracted into five solubility fractions, as described by Landry and Moreaux (1970), and measured by the microkjeldahl method. The proteins

found in fraction I are called albumens and globulins, fractions II and III are called prolamines, and fractions four and five are called the glutelin and glutelin-like proteins. In addition, a 0.1 percent sodium hydroxide solution was used to extract a final glutenin-like fraction. Non-protein nitrogen (NPN) was determined from the fraction I nitrogen which remained in solution following precipitation of the proteins with 10 percent trichloracetic acid (TCA).

RESULTS AND DISCUSSION

Response of Lines In Stability Study 1

The stability parameters for all lines are presented in Tables A.5 to A.8 for protein precent, estimated lysine as percent of protein, estimated lysine as percent of sample, and grain yield. The responses of four lines, *viz*: IS.11758 and P-721 (high lysine mutants), Q.50662 (a bulgy line), and CSH-1 (a normal lysine hybrid) are presented graphically in Figures 4.1 to 4.4.

Protein Percent (Figure 4.1) The linear regression lines of four genotypes onto the environmental index for protein percent are shown in Figure 4.1, along with the stability parameters for those lines and the R^2 values which measure the proportion of the variation in a genotype which was accounted for by linear regression. The broken mean line is the plot of the genotype set means at each environment.

When evaluated across environments, the protein levels of IS.11758, P-721 and Q.50662 were higher than the genotype set mean, while the protein level of CSH-1 remained lower than the mean. The b values of P-721, Q.50662, and CSH-1 were all close to 1, indicating that their linear responses were similar to the genotype set mean. Although IS.11758 had a slightly lower b value, which indicated that the protein level in this line tended to be more constant across environments, the deviation mean square value which was associated with IS.11758 was much larger than for the other lines. This large deviation mean square indicated that linear regression was describing poorly the actual environmental response of protein percent in this line.

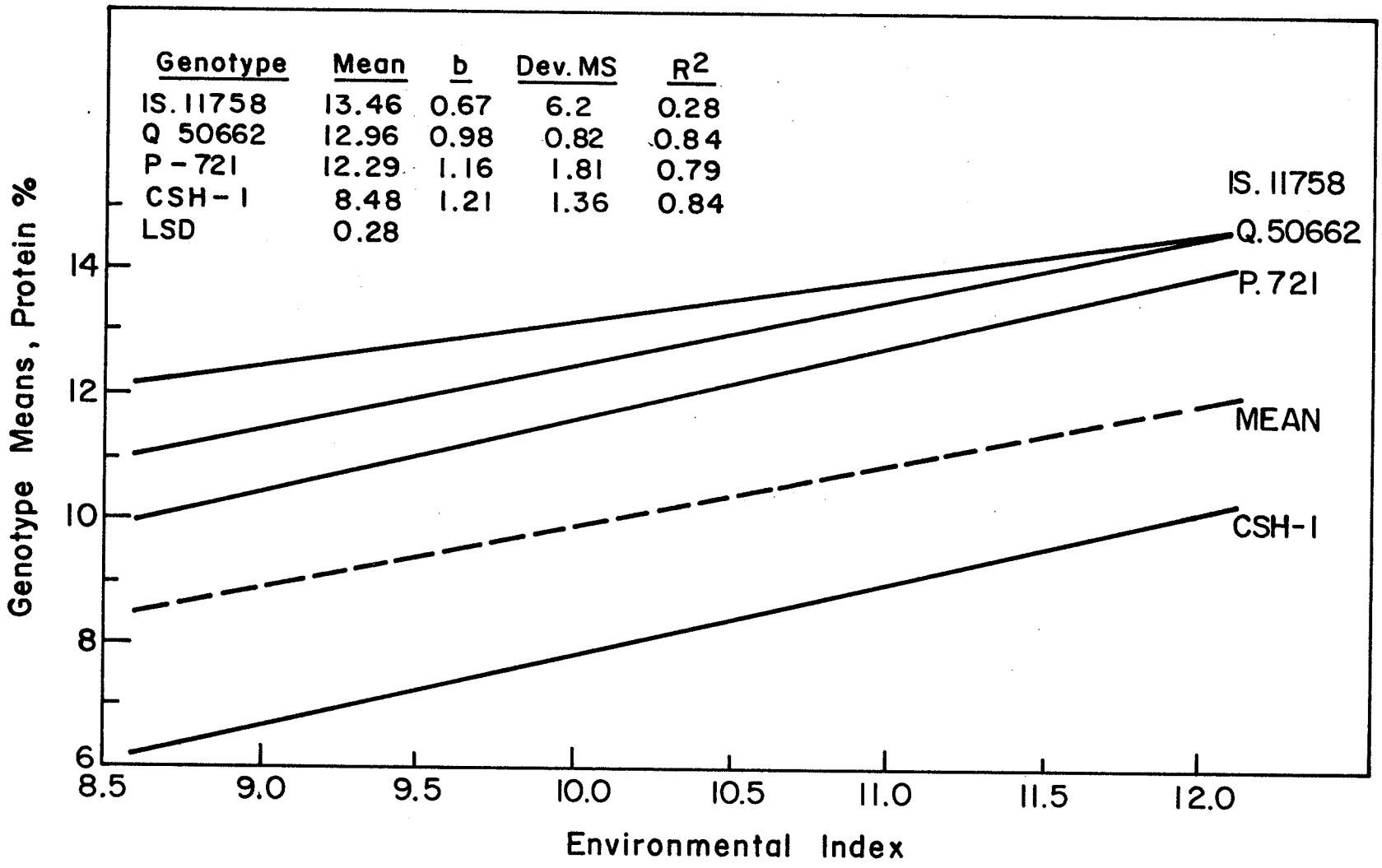

Figure 4.1. Regression of protein percent in 4 of 22 entries tested across 12 environments during 1977 cool dry season. LSD = Least significant difference at 0,05 probability level.

The R^2 values were inversely related to the deviation mean squares, and indicated that linear regression accounted for most of the variation in P-721, Q.50662 and CSH-1. Both R^2 and deviation mean square values are measures of goodness of fit of a genotype response to linear regression. The deviation mean square value will be used henceforth as it is independent of the b value, while the R^2 value decreases as the b value approaches 0.

The similarity in b values of these lines would indicate that genotype by environment interaction was low for protein percent in these lines. Similarly, in Table 3.3, genotype by environment variation was found to be relatively lower for protein levels than for either estimated lysine or grain yield.

Estimated Lysine as Percent of Protein. The negative b value for P-721 (Figure 4.2) was strikingly different from that of the other lines. This negative b value indicated that P-721 was producing lower estimated lysine values in environments where the other genotypes were producing higher estimated lysine. It was earlier pointed out that the environments which produced high estimated lysine were characterized by low protein levels (Tables 3.5 and 3.6). In these environments, P-721 produced estimated lysine levels not significantly higher than those of CSH-1 or of the genotype set mean. The estimated lysine means of both P-721 and Q.50662 were both much lower than that of IS.11758. The b value of Q.50662, however, was close to 1, so that this genotype appeared to maintain its level of estimated lysine over environments. The higher deviation mean square for estimated lysine associated with IS.11758 indicated that linear regression was inadequately describing the actual response of this line.

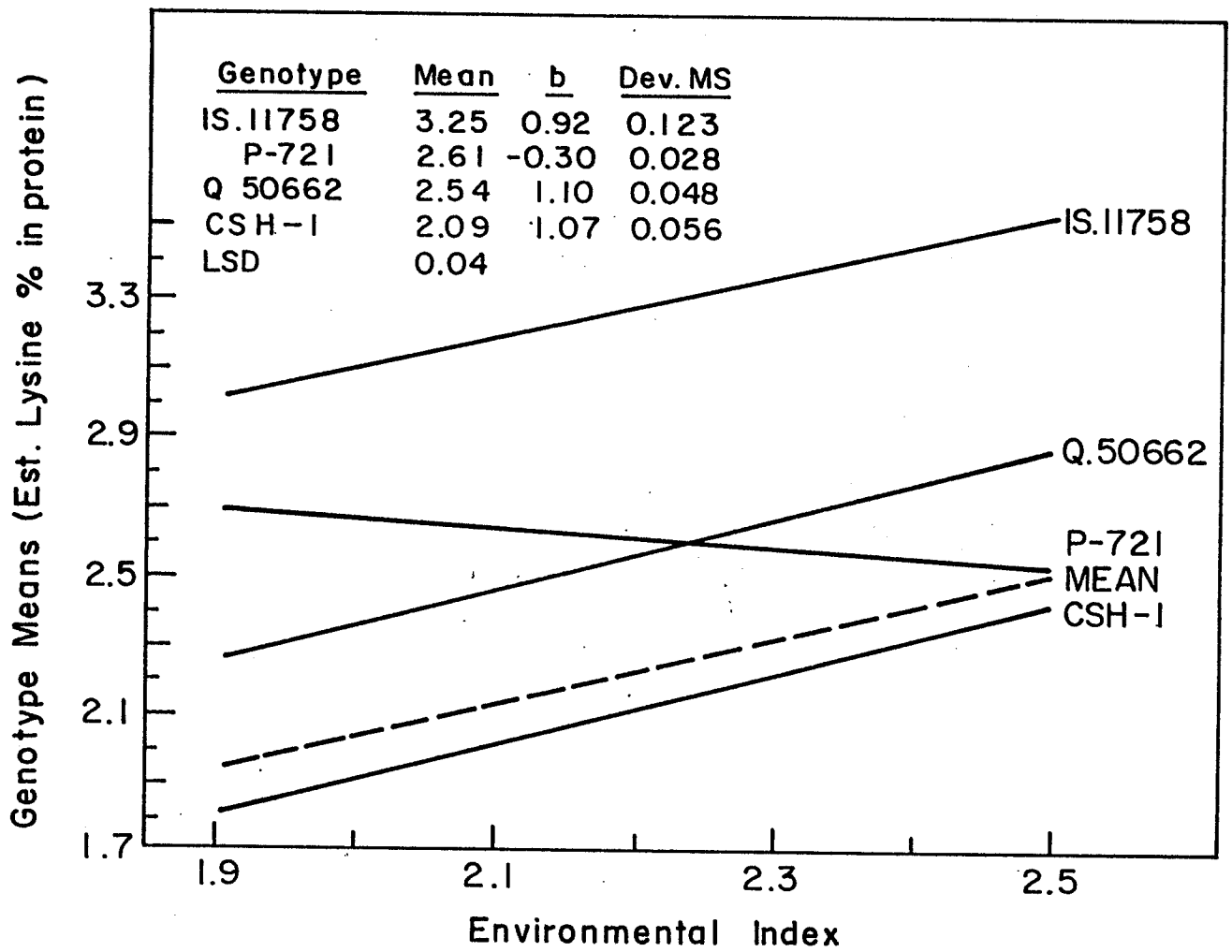

Figure 4.2. Regression of estimated lysine percent in protein in 4 of 22 entries tested across 12 environments during 1977 cool dry season. LSD = Least significant difference at 0.05 probability level.

Estimated Lysine as Percent of Sample (Figure 4.3). The response of some lines was different in the analysis of the cool dry season environments compared with analysis of all 30 environments even though the genotype set means were the same. Therefore, the 30-environment set was used for analyzing this character, but with the stability parameters for IS.11758 from the 1977 cool dry season environments included. Estimated lysine as a percent of sample is a product of estimated lysine in protein, and protein percent. Changes in protein percent tended to influence this character more than did changes in lysine as a percent of protein, so that the environments with the lowest genotype set means for estimated lysine percent in the sample also had the lowest protein percent (Tables 3.5 and 3.6). Although the mean for P-721 was higher than the genotype set mean, the regression line for P-721 dropped to near the genotype set means when the environmental index was low (or when protein percent was low). The regression line for IS.11758 also dropped where the environment index was low, but IS.11758 maintained its superiority over the other entries in all environments.

Grain Yield (Figure 4.4). Although the mean grain yields of the three high and moderately high lysine lines were not significantly different from each other, their b values indicated very different responses over environments. The linear regression line for grain yield was low for IS.11758 and Q.50662 where the environmental index was low, but rose quickly in "better yielding" environments to produce estimated grain yields equal to the genotype set mean when the environmental index was high. The linear regression line for P-721, however, was low

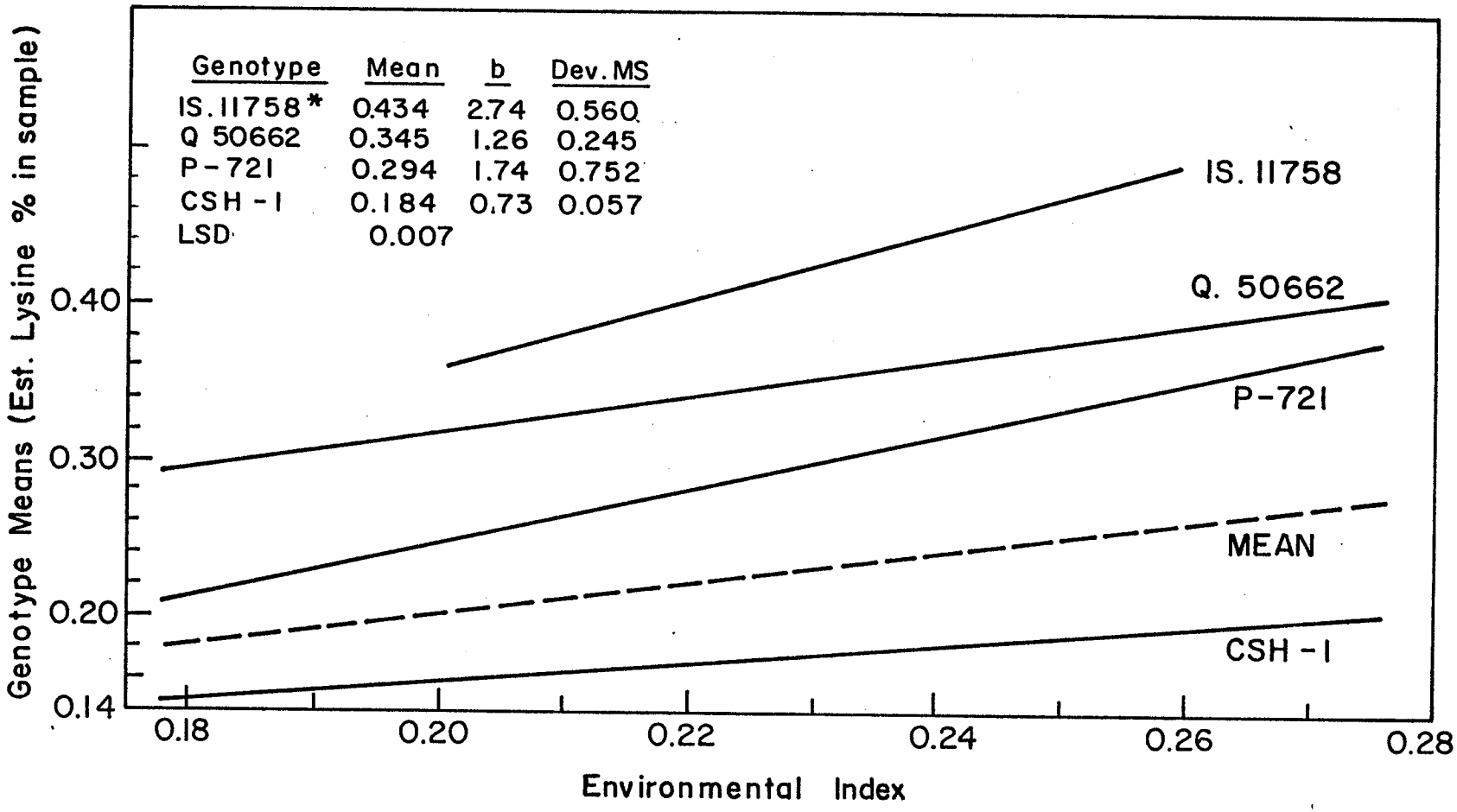

Figure 4.3. Regression of estimated lysine percent in sample in 3 of 18 entries tested across 30 environments. * IS.11758 was tested over 12 environments in the 1977 cool dry season. LSD = Least significant difference at 0.05 probability level.

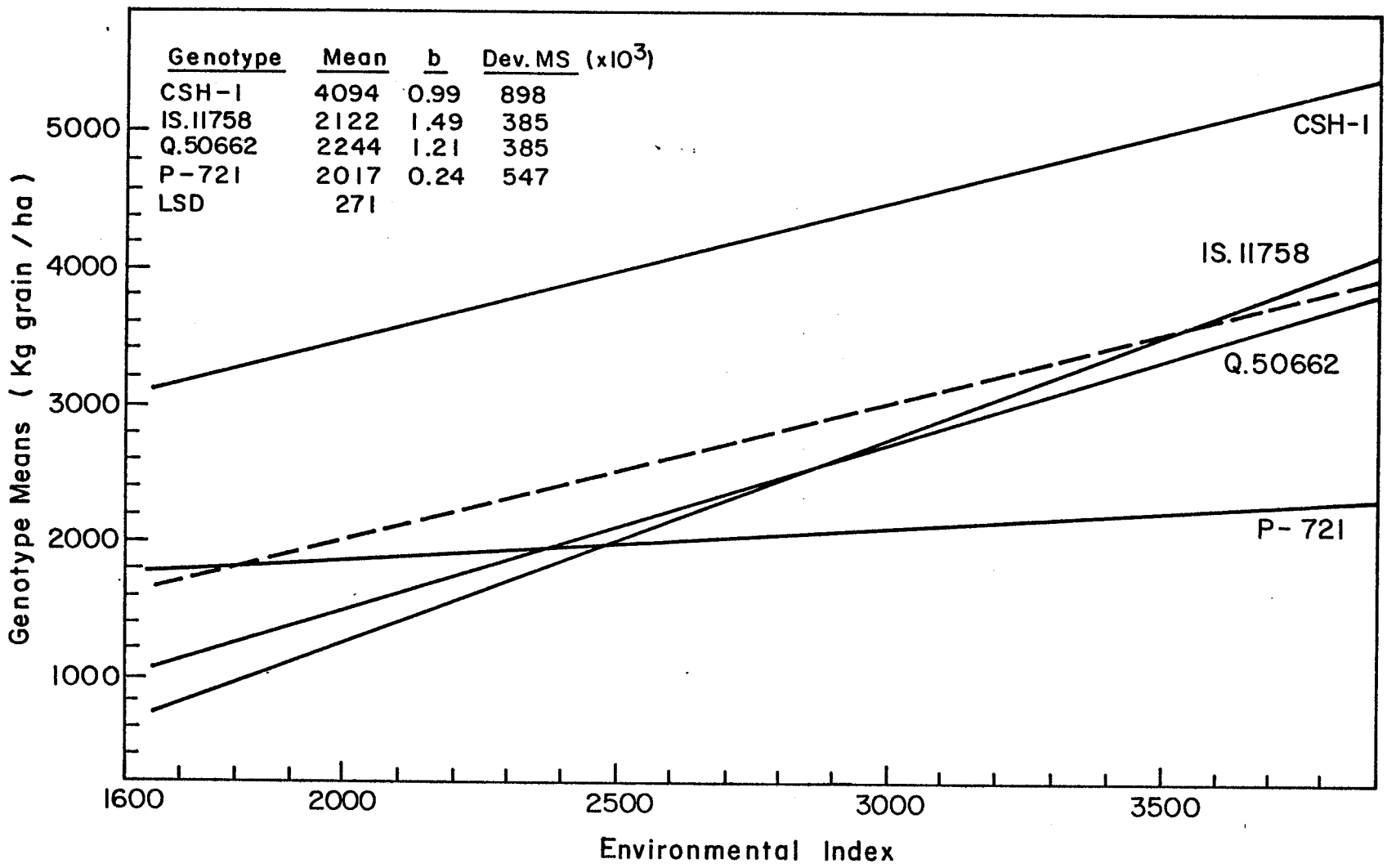

Figure 4.4. Regression of grain yield in 4 of 22 entries tested across 12 environments during 1977 cool dry season
LSD = Least significant difference at 0.05 probability level.

across all environments, and was not responsive in better yielding environments. The CSH-1 hybrid appeared to have high and stable yield, with a mean grain yield of almost twice that of the moderately high or high lysine lines and a b value close to 1.

Correlations Between Estimated Lysine and Protein Percent

Stability analysis indicated that the high lysine mutants had a different environmental response, both compared with one another and compared with the normal lysine hybrid CSH-1. The different correlations across environments between estimated lysine (percent in protein) and protein percent in these genotypes (Figure 4.5) appeared to be related to some of these different responses. The negative correlation ($r = -0.77$) found in CSH-1 indicated that estimated lysine was higher in environments where protein percent was lower. The positive ($r = 0.49$) correlation for P-721, on the other hand, indicated that estimated lysine values decreased in environments where protein percent also decreased, while the lack of correlation ($r = 0.11$) for IS.11758, indicated that changes in estimated lysine values were not related to changes in protein across environments. The response of these 3 lines in the stability analysis had produced a similar interpretation.

Response of Lines in Stability Study 2

Although the estimated lysine in P-721 dropped to near-normal levels in low protein environments, and P-721 produced low grain yields even in high yielding environments, it may be possible to select more promising high lysine lines from crosses with P-721. To examine this possibility, data from four F_4 bulked lines, derived from crosses with P-721 and selected both for agronomic performance and high DBC/P ratios, are

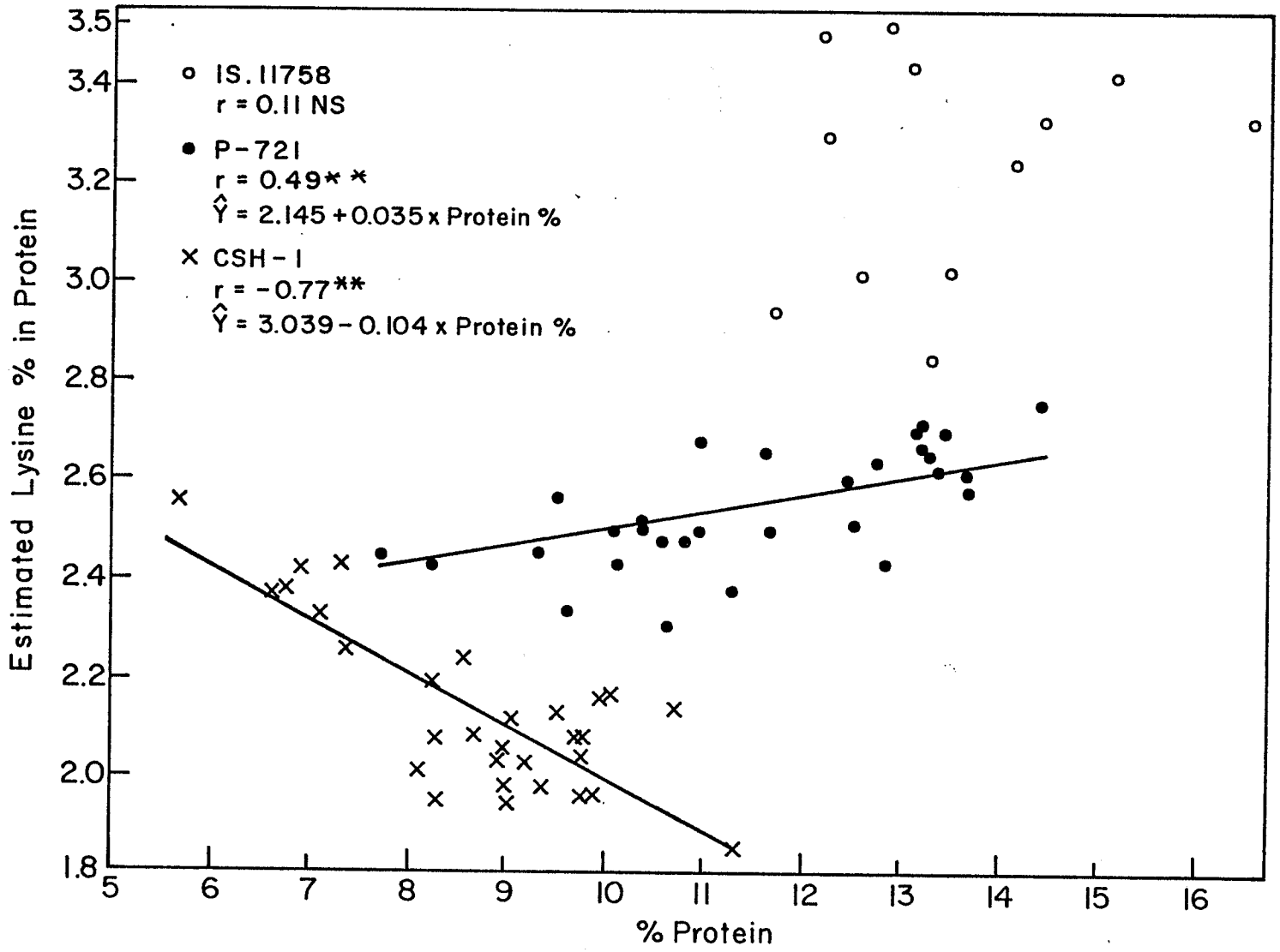

Figure 4.5 Correlation between estimated lysine percent in protein and protein percent in 3 sorghum lines. IS.11758 was tested in 12 environments during 1977 cool dry season; P-721 and CSH-1 in 30 environments over 4 seasons. **Significant at P < .01. NS = Non-significant.

presented in Figures 4.6 to 4.8.

The data show that the four lines produced equal or slightly higher means of estimated lysine (percent in protein) than did the P-721 parent (Figure 4.6). The b values of the derived lines ranged from being more positive to more negative (.43 to -.42) compared with P-721 (-.16). The upper limit of this range, however, was well below the b value of CSH-1 (1.96), and consequently estimated values of lysine in any of the P-721 derived lines were approximately equal to CSH-1 at environments where the index for estimated lysine was high. (The higher b values for CSH-1 as compared with the first stability study was likely due to the different genotype set used, as a greater proportion of the lines in stability study 2 produced high estimated lysine.)

An examination of the response in the same lines for estimated lysine percent in sample (Figure 4.7) again indicated that responses were similar to those found in the first stability study. In environments with a low index, there was little difference between the values of CSH-1 and either P-721 or the derivatives of P-721. However, there were two derivatives, PxQ.4-D and PxQ.8-D for which the b values were somewhat closer to 1 and maintained estimated values of lysine percent in sample above the mean value over all environments.

The grain yields of the four derivatives of P-721 (Figure 4.8) were much lower than that of CSH-1, and were very similar to P-721, both in mean yields and in response over environments. The two lines PxQ.4-D and PxQ.8-D, which maintained higher estimated lysine values, also produced slightly, but not significantly, higher mean grain yield.

Therefore, this group of four high lysine derivatives of crosses

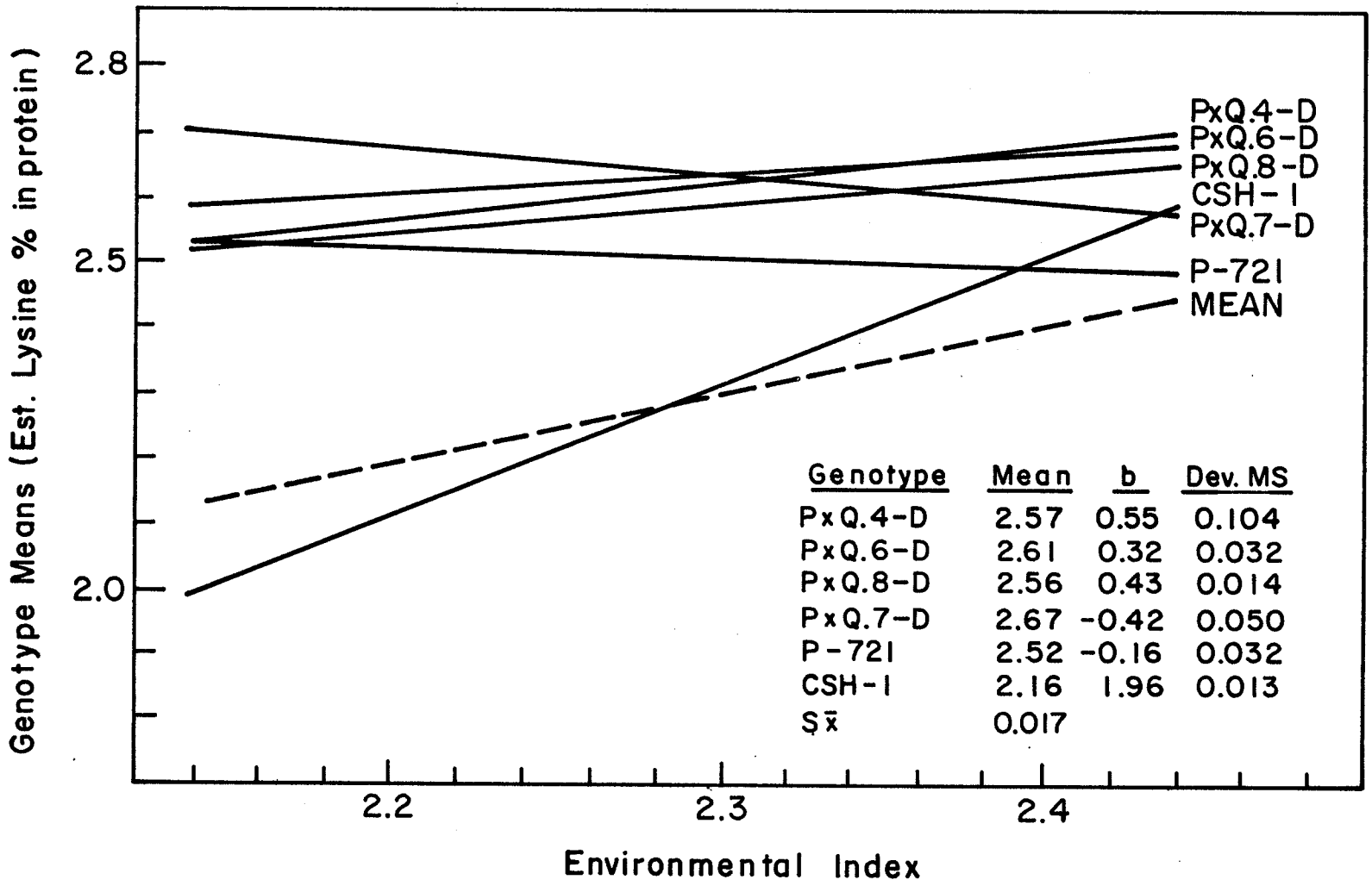

Figure 4.6. Regression of estimated lysine percent of protein in F_4 bulks from crosses with P-721 which produced high estimated lysine. Tested with 23 entries across 12 environments in 1978 warm wet season. $S\bar{x}$ = standard error of the mean.

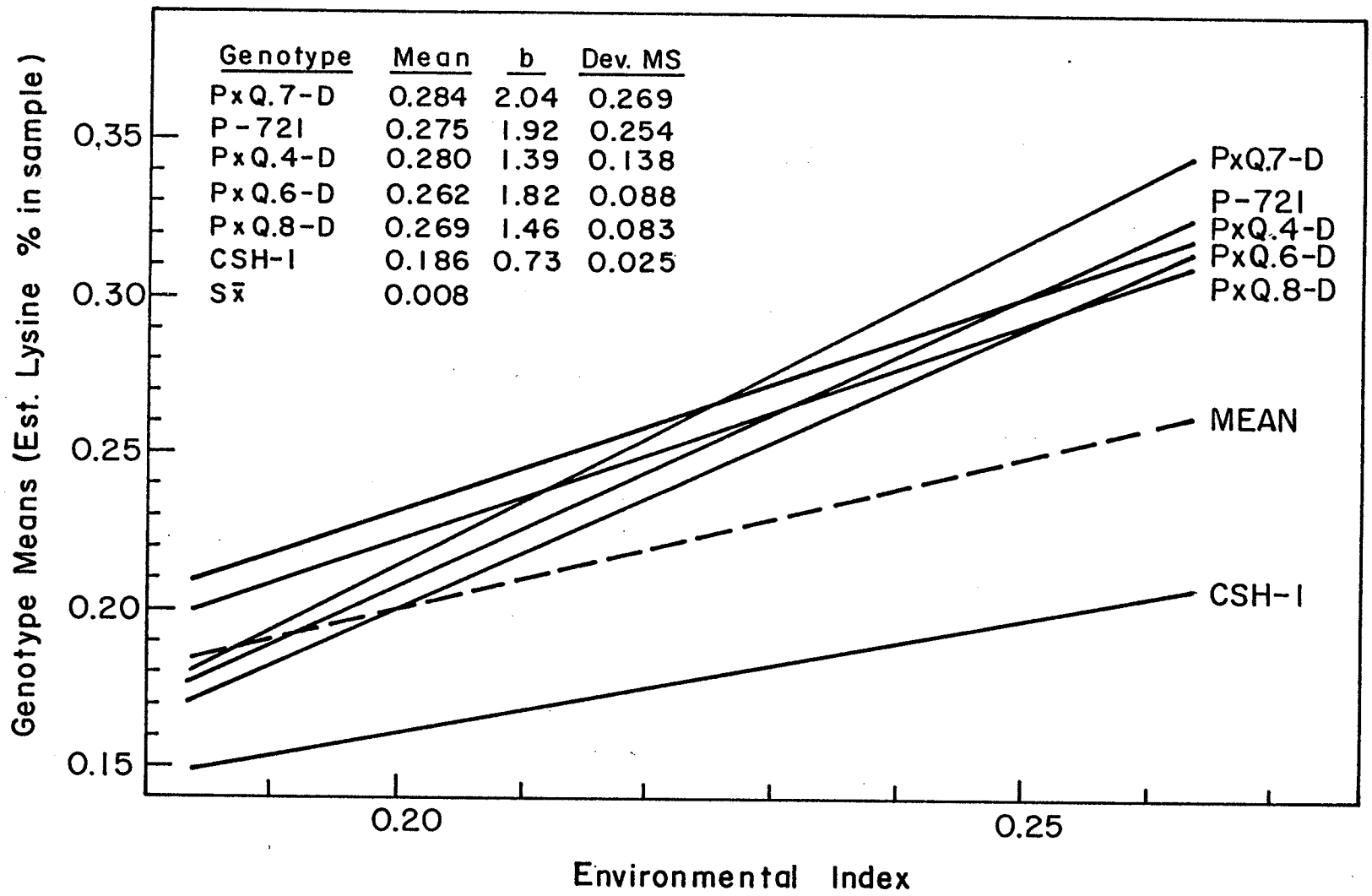

Figure 4.7. Regression of estimated lysine percent in sample in F_4 bulks of crosses with P-721. Tested with 23 entries across 12 environments in 1978 warm wet season. $S\bar{x}$ = standard error of the mean,

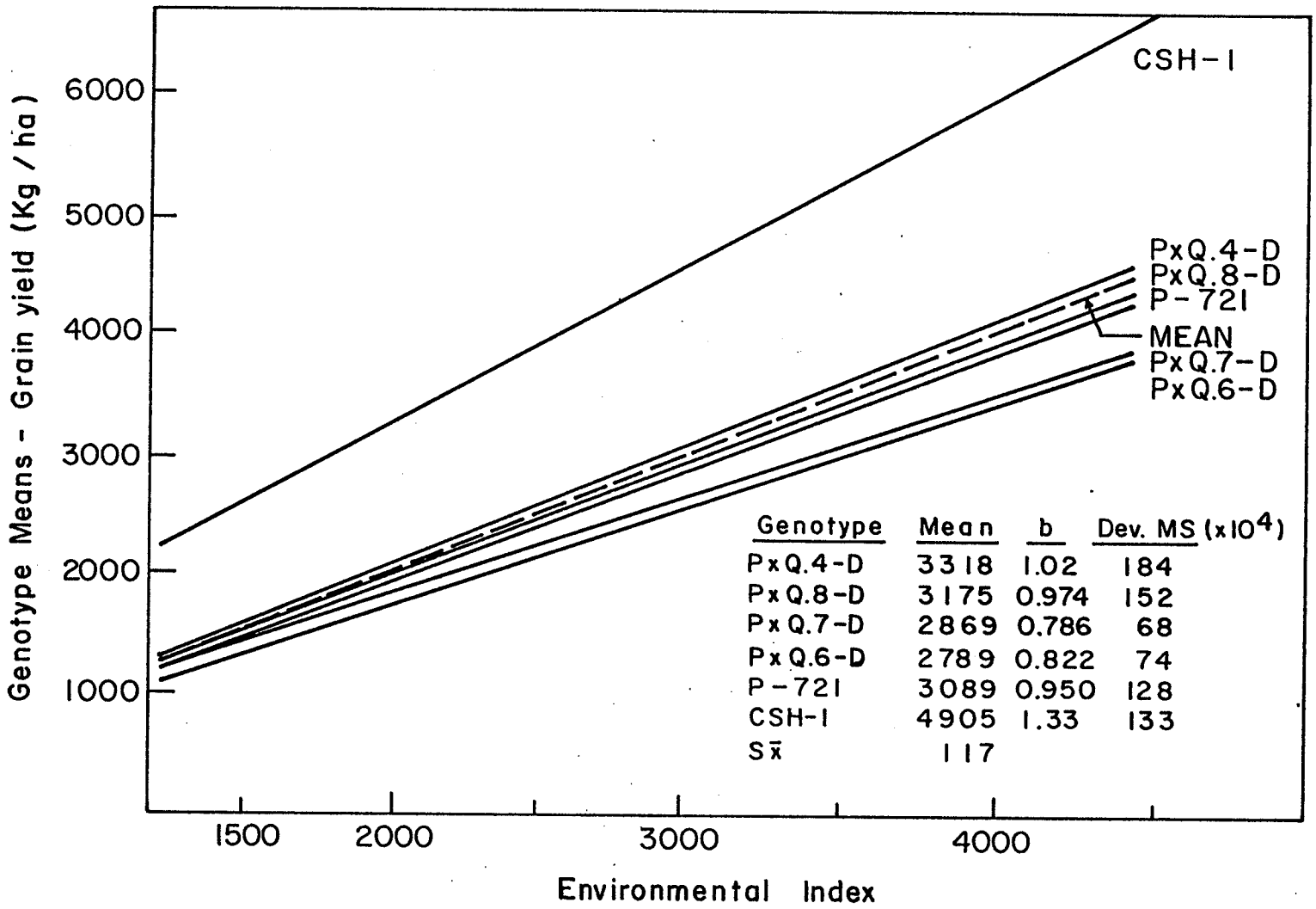

Figure 4.8. Regression of checks and F_4 bulks from crosses with P-721 which produced high estimaed lysine. Tested with 23 entries across 12 environments in the 1973 warm wet season. $S\bar{x}$ = standard error of the mean,

with P-721, had environmental responses similar to those of their P-721 parent. However, two of these lines produced high mean estimated lysine levels and maintained a level of estimated lysine somewhat above that of P-721 across environments. In spite of selection for agronomic appearance, these lines did not produce significantly higher grain yields compared with P-721.

Examination of Single Head Samples

The different environmental responses in P-721 compared with other entries has been found using whole grain samples. To determine whether these changes were also occurring in the endosperm, kernel samples of single heads of CSH-1 and P-721 from a HN and a LN environment were selected which had seed weight, protein and estimated lysine values similar to their respective treatment means. Endosperm, pericarp and embryo components in these samples were separated. Values for protein percent and lysine percent in protein in both whole kernel and endosperm samples are shown in Figure 4.9. Whole seed lysine was determined using the DBC/P regression estimate, but the endosperm lysine determinations were made with the Beckman 120c amino acid analyzer. The higher protein levels in the high nitrogen treatments for both P-721 and CSH-1 was clearly due to the difference in the endosperm protein level. It was also apparent that the differences in the whole seed estimated lysine levels were largely due to differences in the lysine level in the endosperm. The higher lysine levels in the whole seed samples were likely due to the effect of the embryo which has a high level of lysine (Shoup, 1969), and could be expected to have the greatest effect in increasing lysine levels where the endosperm lysine is low, as appeared

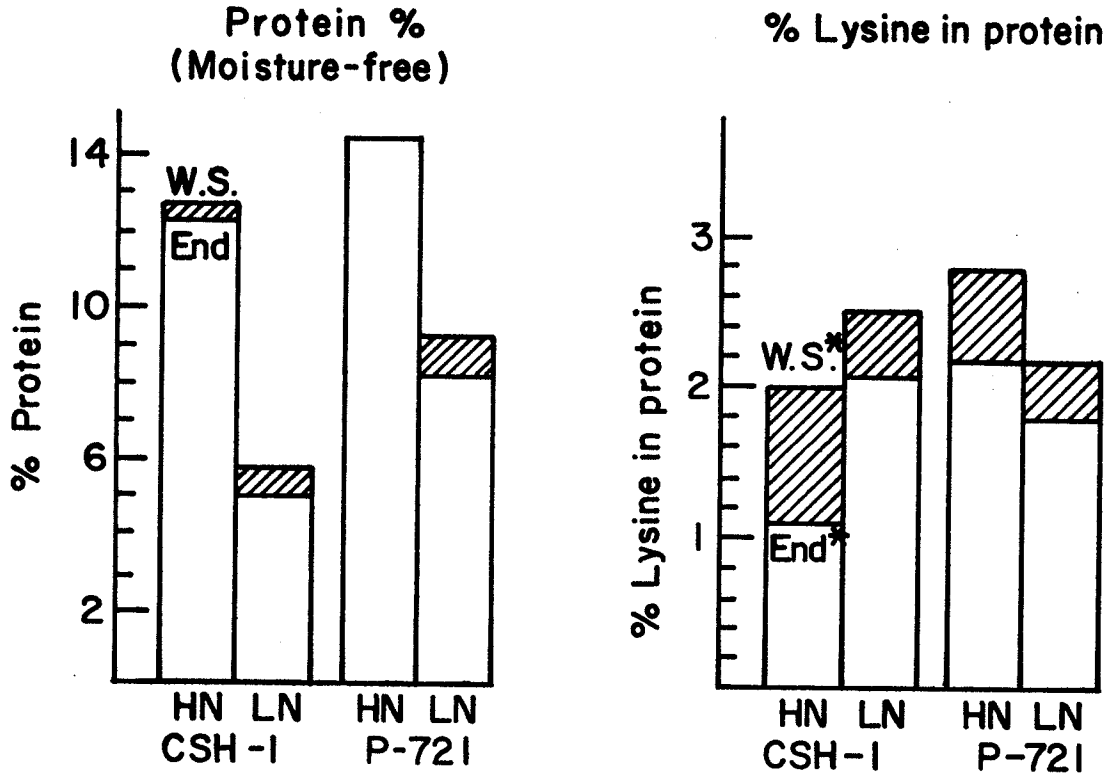

Figure 4.9. Protein % and Lysine % in protein in single heads of P-721 and CSH-1 from high (HN) and low (LN) nitrogen plots in low management black soil during 1977 dry (rabi) Season.

*WS = Whole grain lysine estimated by DBC/P ratios.

**End = Endosperm lysine analyzed by Beckman 120c amino acid analyser.

to be the case with the CSH-1/HN sample.

The nitrogen in the four endosperm samples was then successively extracted into six solubility fractions. The results of this fractionation are shown in Table 4.2. Of special interest are the proportions of extractable nitrogen in fractions II and III. These fractions, known as prolamine or kafirin proteins, function as storage proteins and were shown to have very low concentrations of lysine (Nelson, 1979). The lower level of prolamine in the P-721/HN sample compared with the CSH-1/HN sample was likely due to the effect of the high lysine gene in P-721. The prolamine in fractions II and III dropped from 44.7% (14.5 + 30.2) in CSH-1/HN, down to 33.2% in P-721/HN. Guiragossian *et al.* (1978) found that prolamines accounted for 50% of the nitrogen in normal sorghum and 25% in P-721 when grown at Purdue University.

The lower level of prolamine in the CSH-1/LN sample (32.5%) compared with CSH-1/HN (44.7%) apparently resulted from limited nitrogen availability during seed filling in the low nitrogen sample. However, the P-721/LN sample produced a slightly higher level of prolamine (40.0%) than did P-721/HN (33.2%). It would appear that the P-721 mutant was not effective in reducing the proportion of prolamine protein in conditions where nitrogen was limiting. This finding appeared to be supported both by the lower lysine percent found in the protein of the P-721/LN sample compared with the P-721/HN sample (Figure 4.9), and by the stability analysis which indicated that the estimated lysine percent in protein in P-721 was slightly lower in environments in which nitrogen was limiting and in which protein percent was lower.

The level of prolamine in the CSH-1/LN sample (32.6) was lower than

TABLE 4.2. Fractionation of endosperm proteins in individual heads of P-721 and CSH-1 from high (HN) and low (LN) nitrogen treatments in low fertility black soil during 1977 cool dry season.

	Fraction	CSH-1		P-721	
		HN	LN	HN	LN
	Percent of soluble N				
I	(albumens and globulins)	4.1	10.7	7.2	6.6
II	(prolamines)	14.5	5.2	10.6	13.2
III	(cross-linked prolamines)	30.2	27.4	23.0	26.8
IV	(glutelin-like)	4.5	6.7	3.2	5.4
V	(glutelin)	36.8	41.6	40.6	39.6
VI	(glutelin-like, alkali-soluble)	9.9	8.5	15.4	8.4
	Total N extracted	94.6	95.7	93.1	92.5
NPN[@]	as % N	1.2	3.3	2.7	3.0
NPN	as % of sample	.023	.018	.063	.039

[@]Nitrogen in fraction I which was not precipitated with 10% TCA.

that in P-721/LN (40.0), which was in accord with the slightly higher level of lysine found in the CSH-1/LN sample compared with that in P-721/LN (Figure 4.9). The stability analysis indicated no real difference in the lysine levels of these two lines in environments where nitrogen was most limiting and protein percent was lowest (Figures 4.2 and 4.6).

Non-protein nitrogen (NPN), when expressed as a percentage of N extracted, was highest in the CSH-1/LN sample. However, when expressed as a percent of the sample, the CSH-1/LN treatment produced the lowest value, as expected if nitrogen in the plant was limiting during grain filling. Both the P-721 samples produced higher levels of non-protein nitrogen in the sample, compared with CSH-1. Increased amounts of NPN have been previously reported for the high lysine maize and barley mutants (Brandt, 1976; Gupta *et al.*, 1977). In all samples, however, non-protein nitrogen accounted for only a small proportion of the total nitrogen.

The interaction between high lysine and normal lysine barley and maize lines at moderate to high levels of N (Anderson and Koie, 1975; Zink and Wilberg, 1976) appeared somewhat similar to the interaction between P-721 and CSH-1 sorghum at moderate to low rates of applied N. In both cases, increases in applied N resulted in less change in both prolamine levels and lysine (as percent of protein) levels in the high lysine lines than in the normal lysine lines. The slight decrease in lysine levels found with P-721, contrasted with the slight increase or no change in lysine reported for high lysine maize and barley. This could be due to the different levels of nitrogen applied in the different experiments. Protein percent increased in both high and normal lysine lines in all experiments with increases of N.

In the high lysine lines of all these crops, the high lysine gene appeared to reduce the proportion of prolamine protein, so that prolamine remained relatively constant across environments, while in normal lysine lines, the proportion of prolamine protein increased in environments where N was abundant, and decreased where N was limiting. This produced large differences in lysine between the high and normal lysine lines where N was abundant, but little or no difference where N was limiting.

The failure to find any yield improvement in high lysine derivatives of P-721 (Figure 4.8) and the lack of response for grain yield across environments (Figure 4.4) in the P-721 parent may be related to the reduction in the sink size for N, caused by the reduction in prolamine. Tsai *et al.* (1978) observed a close correlation between zein (or prolamine) accumulation and grain yield in maize, and felt that "zein and glutelins may serve as a functional N sink in the kernel to affect starch accumulation, kernel weight and yield." This might explain the difficulty in increasing yields in the high lysine mutants, in which prolamine is reduced. A possible way to circumvent the problem was suggested by Axtell (1979) who felt that grain yield in high lysine derivatives of P-721 might be increased by selecting for greater numbers of kernels per head, rather than selecting for heavier kernels. However, the apparent failure of this high lysine source to express higher than normal lysine levels in environments where N was limiting appears to make the value of P-721 doubtful in many semi-arid tropical environments.

In the stability analysis, the two bulgy lines, Q.50662 and Q.50687, appeared to have high protein levels as well as mean estimated lysine

levels equal to or higher than those of P-721, and to maintain a relatively high lysine level across environments. However, the kernel weights of the bulgy lines were quite low, and it appeared that actual lysine levels in the bulgy lines may be slightly lower than that of P-721 (Table 2.3). These two lines were also tall, with fragile stalks, and appeared to be better adapted to the shorter days of the cool dry season. These problems might be overcome by breeding and selection, but it is doubtful if this source of high lysine offers any improvement over the existing sources.

The response of the Ethiopian high lysine mutant, IS.11758, was characterized by high protein and high estimated lysine levels over environments. However, the variation of protein and estimated lysine in this line was not well described by linear regression. This may simply mean that the protein and lysine levels do not correspond with levels of other genotypes over environments. Grain yields were very low in "low yielding" environments where the environmental index was low, but increased rapidly until they were equal to the mean in "high yielding" environments.

In Manuscript 2, it was shown that the *hl* high lysine gene had been lost in the plump-seeded selections from crosses with the Ethiopian *hl* mutants. However, it might be worthwhile to take a second look at the IS.11758 parent. Table 4.3 illustrates the performance of IS.11758 in the highest yielding environment, where it produced grain yield equal to the mean at that environment, and produced more protein per hectare, and more lysine in the sample than did P-721. Although kernel weight remained low across environments (Figure 4.10), which appeared to be

TABLE 4.3. Performance of 3 genotypes at the highest yielding (high management red soil-high nitrogen) environment during the 1977 cool-dry season.

Genotype	Grain yield (kg/ha)	Protein yield (kg/ha)	Est. lysine (% of sample)
CSH-1	5617	495	.178
IS.11758	3929	559	.374
P-721	2602	344	.351
Mean	3942	421	.219
LSD*	1100	120	.02

*LSD = Least significant difference at 0.05 probability level.

associated with the dented seed, this genotype appeared to have the capacity to produce very high numbers of seeds per head in "high yielding" environments (Figure 4.11).

It was not surprising that IS.11758 produced high yields in only a few environments, since this high lysine mutant has been found only in isolated valleys in the Ethiopian Highlands. It is possible that selection by Ethiopian farmers both for larger heads and for the dented kernel character, which they associated with the sweet taste of this seed during the dough stage of ripening, resulted in the very high seed number in this line. It would appear that the sink for N, although small on an individual kernel basis, is large when considered on a per plant basis.

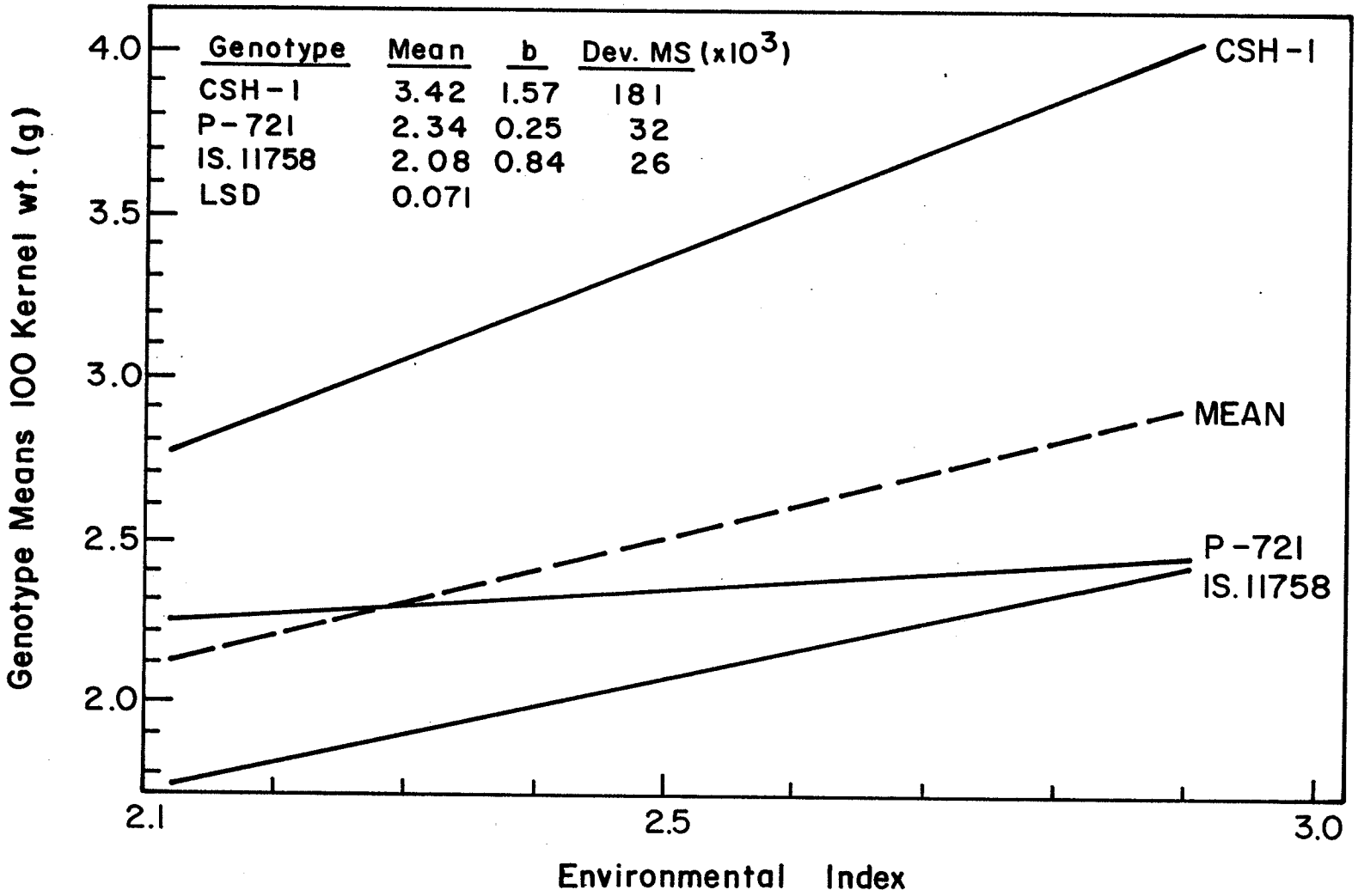

Figure 4.10. Regression of kernel weight in 3 of 22 entries tested across 12 environments during 1977 cool dry season. LSD = Least significant difference at 0.05 probability level.

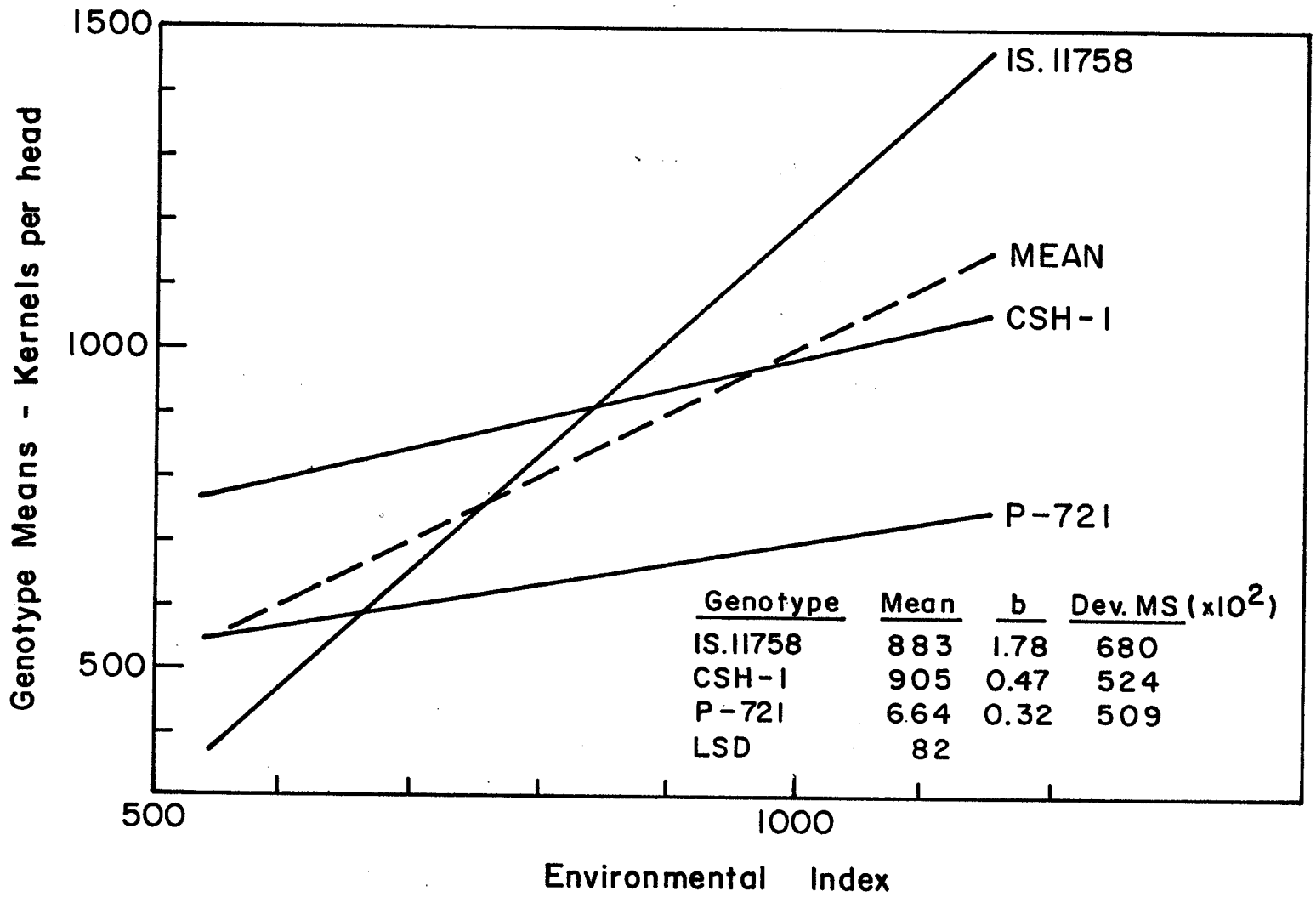

Figure 4.11. Regression of number of kernels per head in 3 of 22 entries tested across 12 environments during 1977 cool dry season. LSD = Least significant different at 0.05 probability level.

CONCLUSIONS

The high lysine mutant, P-721, had little nutritional advantage over the normal lysine hybrid CSH-1 under low N conditions, but the differences became more pronounced with higher fertility. The protein levels dropped in low N conditions with both lines. This drop in protein was accompanied by a rise in estimated lysine as a percent of protein in CSH-1, whereas the lower protein levels were accompanied by a slight decline in estimated lysine levels in P-721. As a result, levels of estimated lysine in the sample in CSH-1 approached those of P-721 under low N conditions.

This different response was due to changes in protein and lysine levels in the endosperm in these lines. Fractionation of the protein in endosperm samples revealed that changes in the prolamine or kafarin levels could account for the different environmental response in lysine in CSH-1 compared with P-721. It appeared that both prolamine and lysine levels remained more constant in P-721 compared with CSH-1 as the level of applied N increased.

Selections made for agronomic appearance and estimated lysine among crosses with P-721 produced some derivatives which were significantly higher in estimated lysine, and which maintained their nutritional advantage over environments somewhat better than the P-721 parent. However, levels of estimated lysine as percent of protein in these derivatives were no higher than that of CSH-1, in conditions where N was low. Grain yields of the derivatives were very similar to that of the P-721 parent, which was found to be low and not responsive in higher

yielding environments. The value of P-721 would appear doubtful in many semi-arid tropical environments.

Two lines with a bulgy appearance of the kernel possessed moderately high lysine levels. These lines possessed several undesirable traits, and did not appear to have any advantage over the two other high lysine sources.

The environmental response of IS.11758 was different from either the P-721 or the normal lysine response. Levels of both protein and lysine in IS.11758 were high and were unrelated to each other over environments. Consequently the nutritional advantage remained well above that of normal lines, including CSH-1. IS.11758 was capable of producing moderately high grain yields in the most favourable environments, in spite of its light, dented kernels, by compensating with large numbers of kernels per head.

GENERAL DISCUSSION

The relevance of breeding sorghum for improved nutritional quality, can be examined in both a short term and a long term context.

At the present time, the objective of incorporating the high lysine genes into all breeding material is questionable for several reasons. Nutritional studies have indicated that cereals with improved protein quality can benefit young children from among the lowest income groups (Singh and Jain, 1977), but it has been difficult to demonstrate that the rest of the population would derive any benefit. The high lysine lines with the P-721 gene, as well as the Ethiopian *hl* gene, have been associated with reduced grain yields (Mohan, 1975; Manuscript 4; Axtell *et al.*, 1979). Floury endosperms in these lines have been associated with poor milling and processing quality (Andersen *et al.*, 1977; Murty, 1979). Attempts to produce either high yielding lines with plump kernels (Manuscript 2), or kernels with vitreous endosperms have not been successful in maintaining the lysine levels of the high lysine parents (Ejeta, 1979; Meckenstock, 1979). Lines which appeared to possess the P-721 gene had little nutritional advantage over normal lysine lines in environments where soil nitrogen availability was low. Such environments are not uncommon in the semi-arid tropics (Manuscript 3).

Axtell *et al.* (1979) have suggested the direct use of the Ethiopian high lysine lines for production as a grain for weaning food for infants. It should be possible to incorporate a number of improvements into these lines by breeding. Improved adaptation, and a range of maturities could

be selected in progenies of crosses with normal lysine lines. At the same time, selection for the high kernel number, and the dented kernel, characteristic of the *hl* parents, must be maintained. Chemical analysis for protein and lysine and evaluation for weaning food-making properties need only be made of the most promising lines in advanced generations. Such a breeding program could be carried out with a minimum of expensive laboratory support, as the dented kernel would indicate the presence of the *hl* gene.

In the longer term, it can be expected that research efforts will lead to improved management of much of the sorghum grown in the semi-arid tropics. The increased use of chemical fertilizer, intercropping with legumes, and soil and moisture conservation measures are likely to increase soil fertility and to produce higher protein levels. However, will the nutritional value of sorghum grain deteriorate due to the lower protein quality which can be expected with higher protein levels? It would appear that the reverse is true. In Tables 3.5 and 3.6, the environments which produced the highest protein levels also produced the highest lysine as percent of sample. Thus, it can be expected that agronomic practices which increase protein levels, are likely to increase the nutritional quality of sorghum, provided that the cultivars remain unchanged.

Negative genotype correlations have been found between yield and protein percent in sorghum (Collins and Pickett, 1972). This would indicate that selection for yield without any selection for protein could be expected to result in varieties with lower protein levels. However, higher yielding oat cultivars which maintained a high protein level,

and higher protein wheat which maintained a high yield level, have been selected (Frey, 1975; Middleton *et al.*, 1954). Recently, Tsai *et al.* (1978) have found a close correlation between the accumulation of zein, which is the major protein fraction in maize kernels, and grain yield in maize. These authors suggested that starch accumulation may be enhanced in kernels with a large sink size for zein. Thus, it would appear to be possible to select plants which are capable of producing increases both in grain yield and protein levels.

The high lysine line, IS.11758, appeared to possess a high protein effect which was independent of the *hl* gene (Table 2.5). Plump-seeded segregants from crosses with IS.11758 were 1.2 to 3.4 percent higher in protein than their normal lysine parents. IS.11758, which possessed a high seed number per head, and produced surprisingly favorable yields even with dented seeds (Table 4.3) might be a useful source of both high protein, and high grain yield.

SUMMARY AND CONCLUSIONS

Reasonably close agreement was found between lysine as percent of protein from Beckman amino acid analysis and estimated lysine values. Estimated lysine was calculated from a prediction equation with two terms, *viz*: dye binding capacity/protein percent (DBC/P) ratio, and protein percent. The correlation between the two methods was very close ($r = 0.95$) in samples from an environment which produced high protein levels and fairly close ($r = 0.88$) in the same lines sampled from an environment which produced low protein. In two of the lines, lysine did not appear to be well estimated by the DBC/P method. Therefore, estimated lysine levels should be confirmed in samples of representative lines being tested.

Lines derived from crosses with the Ethiopian high lysine (*hl*) parents, and selected for plump kernels and high DBC/P ratios, did not contain the *hl* gene. Instead, two independent recessive genes were found in two of the thirteen lines tested, which controlled moderately high lysine levels and a bulgy appearance of the kernel. The other lines did not produce high lysine levels. This evidence suggested that the *hl* gene controlled both a dented kernel character and high lysine, and that selection of plump kernels had resulted in loss of the *hl* gene.

An investigation of environmental responses was carried out using a predominantly normal lysine set of genotypes, and tested across a range of environments in semi-arid tropical India. High lysine levels, almost equal to those found in the second high lysine mutant P-721, were produced by genetically normal lysine lines in environments where protein levels were low. Levels of lysine (as percent of protein) showed

a rather close negative relationship to protein per kernel as well as to protein percent across environments. Conditions of low N availability, which frequently occurred at these environments, produced low protein levels, especially if N was limiting during grain filling. In the high lysine breeding program at ICRISAT, environmentally induced high lysine may have resulted in the selection of single plants, during generation advancement, which subsequently produced lines with normal lysine levels.

The interaction variance between genotypes and environments (σ^2_{ge}) was found to be significant, but was considerably smaller than both genotypic variation (σ^2_g) and environmental variation (σ^2_e) for protein percent, estimated lysine, and grain yield.

Much of the environmental variance was accounted for by interaction between the factors investigated, *viz*: season, soil type, level of management, nitrogen top dressing, and moisture stress. Specific knowledge of conditions during growth at the sites tested was often required to explain this variation. The implications of these findings were discussed.

The high lysine mutant P-721, had little nutritional advantage over normal lysine CSH-1 in environments where N availability was low, but the difference was more pronounced with higher fertility. This difference in response was related to the effect on the prolamine fraction. As soil nitrogen was decreased, the prolamine fraction of the endosperm of CSH-1 was substantially reduced, whereas in P-721 it was slightly increased. P-721 also differed from normal lysine sorghums by producing a positive protein-lysine correlation across environments.

Derivatives of crosses with P-721 were found which produced higher

estimated lysine than the P-721 parent at environments where N availability was low. However, compared with CSH-1, the nutritional advantage of these derivatives was only marginal. Grain yields in both P-721 and its derivatives were low and not responsive to more favourable environments.

The environmental response of the Ethiopian high lysine line IS.11758 was different from that of either the P-721 or normal sorghums. Protein and lysine levels remained high over environments and were uncorrelated with each other. The nutritional value of this line across environments remained well above that of any other genotype tested. This line, which possessed light, dented kernels, was capable of producing moderately high grain yields in the most favourable environments by compensating with a large number of kernels per head. Also revealed in this line was a genetic component for high protein which was independent of the *hl* gene.

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TABLE A-1. Pedigree, generation when bulked, and description of lines in the two-season and irrigation experiments.

Entry	Generation when bulked	Pedigree or Description
		Lines entered in the two-season experiment
Q.50662	F_6	(73 PP_9R* x IS.4562) x 73 PP_9R (PP_3R x IS.11167)
Q.50687	F_8	73 PP_9R x IS.11167
Q.50890	F_8	73 PP_3R (PP_3R x IS.11167)
Q.50902	F_8	"
Q.50999	F_6	(NP_3R x IS.6908) 73 PP_9 x MY (PP_3R x IS.11758)
Q.51008	F_6	"
Q.51056	F_8	(NP_3R x IS.6908) 73 PP_9
Q.51234	F_8	"
Q.50738	F_8	PP_2R x IS.11758
Q.50699	F_8	"
Q.50922	F_8	"
Q.52155	F_8	73 PP_5R (PP_1R x IS.11758)
Q.53220	F_8	PP_2R x IS.11758
CSH-1		A widely grown Indian hybrid
GPR-148		A released Indian variety
CS-3541		"
RS_1 x VGC		A promising ICRISAT line
P-721		High lysine mutant from Purdue University
		Additional lines entered in the irrigation experiment
Q.50678	F_8	PP_2R x IS.11758
M 35-1		A popular Indian Farmers' line
BP-53		A farmers line reported to have high lysine and low protein
IS.11758		Ethiopian high lysine mutant

*PP_9R is a Purdue population with the Ethiopian high lysine source included.

TABLE A-2. Expected mean squares of the components in the two-season experiment at ICRISAT center.

Component	df	Expected mean squares
Season (Y)	1	$\sigma_a^2 + 8\sigma_{gy}^2 + 144\sigma_y^2$
Management (M)	1	$\sigma_a^2 + 8\sigma_{gm}^2 + 144\sigma_m^2$
Y x M	1	$\sigma_a^2 + 4\sigma_{gym}^2 + 72\sigma_{ym}^2$
Soil (S)	1	$\sigma_a^2 + 8\sigma_{gs}^2 + 144\sigma_s^2$
Y x S	1	$\sigma_a^2 + 4\sigma_{gys}^2 + 72\sigma_{ym}^2$
M x S	1	$\sigma_a^2 + 4\sigma_{gms}^2 + 72\sigma_{ms}^2$
Y x M x S	1	$\sigma_a^2 + 2\sigma_{gyms}^2 + 36\sigma_{yms}^2$
Nitrogen (N)	1	$\sigma_a^2 + 8\sigma_{gn}^2 + 144\sigma_n^2$
Y x N	1	$\sigma_a^2 + 4\sigma_{gyn}^2 + 72\sigma_{yn}^2$
M x N	1	$\sigma_a^2 + 4\sigma_{gmn}^2 + 72\sigma_{mn}^2$
Y x M x N	1	$\sigma_a^2 + 2\sigma_{gymn}^2 + 36\sigma_{ymn}^2$
S x N	1	$\sigma_a^2 + 4\sigma_{gsn}^2 + 72\sigma_{sn}^2$
Y x S x N	1	$\sigma_a^2 + 2\sigma_{gysn}^2 + 36\sigma_{ysn}^2$
M x S x N	1	$\sigma_a^2 + 2\sigma_{gmsn}^2 + 36\sigma_{msn}^2$
Y x M x S x N	1	$\sigma_a^2 + \sigma_{gymsn}^2 + 18\sigma_{ymsn}^2$

continued .....

TABLE A-2 - continued

Component	df	Expected mean squares
G	17	$\sigma_b^2 + 16\sigma_g^2$
G x Y	17	$\sigma_b^2 + 8\sigma_{gy}^2$
G x M	17	$\sigma_b^2 + 8\sigma_{gm}^2$
G x Y x M	17	$\sigma_b^2 + 4\sigma_{gym}^2$
G x S	17	$\sigma_b^2 + 8\sigma_{gs}^2$
G x Y x S	17	$\sigma_b^2 + 4\sigma_{gys}^2$
G x M x S	17	$\sigma_b^2 + 4\sigma_{gms}^2$
G x Y x M x S	17	$\sigma_b^2 + 2\sigma_{gyms}^2$
G x N	17	$\sigma_b^2 + 8\sigma_{gn}^2$
G x Y x N	17	$\sigma_b^2 + 4\sigma_{gyn}^2$
G x M x N	17	$\sigma_b^2 + 4\sigma_{gmn}^2$
G x Y x M x N	17	$\sigma_b^2 + 2\sigma_{gymn}^2$
G x M x N	17	$\sigma_b^2 + 4\sigma_{gmn}^2$
G x Y x M x N	17	$\sigma_b^2 + 2\sigma_{gymn}^2$
G x M x S x N	17	$\sigma_b^2 + 2\sigma_{gmsn}^2$
G x Y x M x S x N	17	$\sigma_b^2 + \sigma_{gymsn}^2$
Main plot error	24	σ_a^2
Split plot error	816	σ_b^2
Reps/sites	24	
Total:	1151	

TABLE A-3. Error terms (σ^2) and means ($\bar{x}$) across sites tested in stability investigations.

Site*	100 Kernel wt.(g)		Protein (%)		Est.lys.(% in protein)		Yield(kg/ha)	
	σ^2	$\bar{x}$	σ^2	$\bar{x}$	σ^2	$\bar{x}$	σ^2	$\bar{x}$
	1977 Cool dry season							
HMBW	.028	2.60	0.35	10.6	.006	2.18	393,396	2711
HMBD	.026	2.15	0.37	10.1	.008	2.20	354,916	2246
HMRW	.040	2.87	0.49	10.4	.015	1.99	629,130	3733
HMRD	.050	2.48	0.59	11.5	.012	1.96	759,052	2612
LMB	.033	2.38	0.81	10.1	.011	2.29	305,542	1929
LMR	.032	2.65	0.37	10.4	.009	2.20	452,816	2477
	1978 Warm wet season							
HMB	.016	2.14	0.391	9.6	.032	2.18	397,229	2971
HMR	.018	2.28	1.318	11.1	.016	2.06	342,505	2673
LMB	.018	1.99	0.360	9.0	.008	2.18	1,009,560	3249
LMR	.019	2.03	0.590	11.0	.009	2.18	121,053	1564
His	.050	1.89	0.420	11.6	.010	2.33	1,314,880	4772
Par	.034	2.17	0.700	9.4	.048	2.26	734,849	3844
	1978 Summer season							
Bs	.052	2.41	0.85	10.0	.042	2.16	881,595	3443
	1977 Warm wet season							
HMB	.084	2.04	1.04	11.6	.019	2.13	671,852	2566
His	.054	2.10	0.34	12.6	.012	2.18	249,797	1748
Bs	.026	2.15	1.13	10.4	.009	2.02	368,449	2533

*Site abbreviations: HM, LM = High or low management
B,R = Red or black soil
W,D = Full or restricted irrigation
His = Hissar
Par = Parbhani
Bs = Bhavanisagar

TABLE A-4. Soil chemical properties and available moisture, sampled at the end of grain filling, from sites in stability investigations.

(Y) Season	(M) Management	(S) Soil	(I) Irrig.	Avail. soil N (ppm) Fert N High(HN)	Avail. soil N (ppm) Fert N Low(LN)	Avail. P (ppm)	Exch. K (ppm)	Avail. Na (ppm)	pH 1:2 soil water	E.C. (m.mhos/ cm)
'77 Rabi	High	Black	Wet	47*	46	3.6	184	982	8.4	.34
			Dry	59	64	3.5	191	327	8.3	.19
		Red	Wet	103	103	4.4	96	125	6.3	.06
			Dry	92	103	4.8	113	154	6.4	.04
	Low	Black	Wet	60	64	2.1	364	860	8.1	.04
		Red	"	89	88	2.2	94	100	6.4	.06
'78 Kharif	High	Black	Rainfed	65	64	3.9	222	375	8.4	.22
		Red	"	99	104	4.6	96	114	7.0	.18
	Low	Black	"	75	74	3.3	219	51	8.0	.28
		Red	"	118	123	2.4	97	22	6.2	.15
OTHER SITES										
'78 Summer Bhavanisagar				67	-	4.0	139	83	8.2	.37
'78 Kharif Hissar				76	65	11.7	155	24	8.3	.24
'78 Kharif Parbhani				130	156	32.6	270	63	7.9	.59

*Values are averages of 24 determinations at each site, except Available N values which are averages of 12.

TABLE A-5. Stability parameters for protein percent in lines tested in 12 environments during the 1977 cool dry season.

Line	Mean	b	Dev. MS	R^2
IS.11758	13.47	.67	6.19	.28
Q.50662	12.96	.98	.82	.84
Q.50687	12.33	1.06	2.83	.67
P-721	12.29	1.16	1.81	.78
CS.3541	11.39	1.03	2.49	.68
Q.50902	10.89	1.09	.59	.90
Q.50999	10.84	1.04	.56	.89
Q.51008	10.84	.80	.92	.77
Q.50738	10.76	1.21	1.31	.84
GPR.148	10.61	.93	.55	.87
Q.52115	10.59	1.41	.12	.92
Q.50890	10.46	1.22	1.08	.87
Q.51056	10.44	.86	.83	.81
Q.50678	10.36	.78	.85	.77
Q.51234	10.33	1.11	2.47	.72
Q.50922	9.70	.70	.90	.72
M.35-1	9.21	.92	1.01	.80
Q.53220	9.00	.79	.69	.81
Q.50699	8.99	.83	.25	.89
RS_1 x VGC	8.91	1.01	.38	.91
BP.53	8.65	1.14	1.40	.82
CSH-1	8.48	1.21	1.36	.84
Mean	10.52			
$S\bar{x}$	.01			

$S\bar{x}$ = Standard error of line means.

TABLE A-6. Stability parameters for estimated lysine (percent in protein), tested in 12 environments during 1977 cool dry season.

Line	Mean	b	Dev. MS	R^2
IS.11758	3.25	.92	.123	.43
P-721	2.61	- .30	.029	.30
Q.50662	2.54	1.10	.048	.73
Q.50687	2.43	.85	.027	.73
Q.50999	2.18	1.21	.006	.94
RS x VGC	2.15	1.24	.022	.87
Q.51008	2.14	.85	.049	.91
Q.51234	2.14	1.19	.022	.86
Q.50699	2.13	1.11	.006	.94
Q.53220	2.13	1.05	.009	.91
Q.50678	2.12	.99	.020	.82
Q.50922	2.11	.93	.004	.94
CSH-1	2.09	1.07	.056	.68
BP.53	2.08	1.43	.011	.94
Q.50890	2.08	1.38	.025	.90
Q.50738	2.07	1.02	.013	.88
Q.52115	2.07	1.22	.003	.96
Q.51056	2.06	1.12	.007	.93
CS.3541	2.06	.94	.013	.86
M.35-1	2.04	1.02	.024	.81
GPR-148	2.04	.52	.016	.62
Q.50902	1.99	1.08	.012	.89
Mean	2.20			
$S\bar{x}$	.02			

$S\bar{x}$ = Standard error of line means.

TABLE A-7. Stability parameters for estimated lysine (percent of sample) in lines from 30 environments over 4 seasons.

Line	Mean	b	Dev. MS	R^2
Q.50662	.345	1.26	.024	.54
Q.50687	.309	1.10	.032	.40
P-721	.294	1.74	.075	.42
Q.50999	.232	.89	.004	.74
Q.51008	.231	1.00	.004	.78
CS.3541	.223	.82	.007	.60
Q.52115	.222	1.20	.005	.83
Q.50738	.221	1.31	.007	.80
Q.51234	.218	1.13	.004	.83
GPR-148	.216	.64	.003	.67
Q.51056	.214	1.04	.005	.76
Q.50890	.211	.94	.008	.66
Q.50902	.210	.82	.005	.68
Q.50922	.206	.76	.004	.67
Q.50699	.199	.87	.003	.80
Q.53220	.196	.92	.002	.84
RS x VGC	.192	.79	.002	.81
CSH-1	.184	.73	.006	.61
Mean	.229			
$S\bar{x}$	.005			
Tested only in 12 environments during 1977 cool-dry season.				
IS.11758	.434	2.74	.056	.68
Q.50662	.326	1.45	.011	.74
P-721	.318	1.39	.071	.30
CSH-1	.167	.64	.009	.40
Mean (of 22 lines)	.227			
$S\bar{x}$	.007			

$S\bar{x}$ = Standard error of line means,

TABLE A-8. Stability parameters for grain yield in kg/ha tested in 12 environments during the 1977 cool-dry season.

Line	Mean	b	Dev. MS ($\times 10^3$)	R^2
CSH-1	4094	.99	898	.62
Q.50922	3553	1.36	685	.80
RS x VGC	3507	1.21	1550	.59
Q.53220	3315	1.80	301	.93
Q.50699	3228	1.52	410	.88
BP.53	3057	1.43	422	.86
M.35-1	2754	1.05	726	.68
Q.52115	2704	.83	114	.59
CS.3541	2684	.65	921	.41
Q.51056	2517	.87	504	.67
Q.50678	2410	1.15	463	.79
Q.50738	2400	.80	1415	.41
Q.50999	2301	.90	342	.75
Q.51234	2268	.83	633	.61
Q.51008	2244	.78	580	.60
Q.50662	2244	1.21	849	.72
GPR-148	2161	.58	106	.72
IS.11758	2122	1.49	386	.88
Q.50890	2109	.75	335	.99
P-721	2017	.24	547	.12
Q.50687	1984	.73	475	.60
Q.50902	1979	.75	714	.54
Mean	2621			
$S\bar{x}$	97			

$S\bar{x}$ = Standard error of line means.