PROTEIN AND STRUCTURAL ANALYSIS OF POTENTIAL WIDE-HYBRID CEREALS

by

GENE HARVEY KELTNER

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Approved by

Lynn S. Bates
Major Professor

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

LIST OF PLATES

INTRODUCTION

In recent decades there has been an increased awareness that the potential for human population growth far exceeds the capacity for food production. If the situation is left unchecked it will surely lead to economic and political disaster for both the rich and poor nations. Recent estimates indicate that 300 to 500 million people are undernourished, meaning they do not receive enough food, while another one billion people are malnourished, indicating they receive poor quality food (1).

G. Hardin (2) has portrayed the problem with an analogous situation. He views the wealthy nations with their abundant food resources as living on lifeboats, while the poor nations, unable to feed their now explosive populations, are drowning in the sea. Morally, the wealthy nations cannot ignore any pleas for help from their fellow men, but neither can they totally share their resources (lifeboats), for this would bring about their own demise. There are those who insist that the rich nations can and must cut back on the consumption of food resources. The diversion of those resources to the needy nations would improve the situation in the world in general. Finally, there are others (3) who follow the military logic to let the poorest people die in order to improve the odds of those best able to survive.

In reality there has been a move of the "have-nations" to help the "have-not" nations as evidenced by the existence and activities of numerous international organizations. A sampling of these includes the Ford Foundation and the Rockefeller Foundation, the Cooperative for American Relief Everywhere (CARE), the Catholic Relief Services,

the Lutheran World Relief, the U. N. Food and Agriculture Organization and The World Health Organization, and the U. S. Agency for International Development and its counterparts in other developed countries (4). There is also a series of independent, multinationally funded agricultural research centers, such as the International Rice Research Institute (IRRI), the International Maize and Wheat Improvement Center (CIMMYT) and many more which, through direct or indirect food and seed research and distribution, are aimed at improving nutrition throughout the world.

The philosophy of all these organizations is to share the technology of food production, processing, and preservation as well as the concepts of good health and nutrition as a far more attractive alternative to the sharing of limited food resources. The reallocation of food resources would only postpone the inevitable famine for the needy nations while reducing the food supply safety margin for the wealthier nations. Applying improved food production techniques will give the needy nations a chance to become self-supporting.

Agricultural research to improve the yield and quality of cereal crops and to better adapt them to specific worldwide environmental conditions is a part of improving the technology of food production. Cereal crops are the major staple of the human diet in most parts of the world (5, 6). Worldwide, cereals, tubers, and legumes provide approximately two-thirds of the protein and a major portion of the energy in all diets. The consumption of animal products ranks second, but most of these food sources are consumed by the developed nations. Accordingly, efforts toward increasing food production in developing nations have emphasized increasing present cereal crop yields and

introducing new and improved varieties.

Improving crop yield through breeding research has followed the tradition of crossing within or to closely related species. Plant breeders, looking for desirable improvements such as increased seed set, tillering, and straw strength, select plants exhibiting those qualities. Crosses are then made to obtain the desired traits in a single variety. For example, crossing early maturing varieties of wheat with high yielding types could produce a wheat variety which would yield abundantly in a short growing period. Such a variety could make multiple plantings possible during a growing season and effectively double the yield. Such an example is idealistic and, unfortunately, has not yet been produced.

In more recent years, plant breeders have been seeking to transfer more and different genes from one species to another. Wide-hybrid crossing, so called because distantly related breeding populations are crossed, may be interspecific or intergeneric. The concept has the potential of widening the existing genetic base of present cereal species by incorporating the genes of more distantly related cereals.

Research has been conducted at Kansas State University in cooperation with the International Maize and Wheat Improvement Center (CIMMYT) (7) to further the concepts and possibilities of wide-hybrid crossing among wheat, rye, barley, and oats. This thesis, which is presented in two parts, describes a segment of that research dealing with the protein quality and kernel structure of some of the potential hybrids generated. Part I describes a sectioning technique developed for observing kernel structure and Part II presents data on total

protein determinations, amino acid analysis, and electrophoretic analysis of potential wide-hybrid parents and progeny.

REVIEW OF LITERATURE

Population versus food supply

It is common in the developing countries that the food supply is inadequate in quantity and quality (8, 9) or is poorly distributed at times of greatest need between harvests. Political and economic instability, limited transportation and storage, limited usable land, and climatic catastrophes may produce these situations but the cause may also be traced to retarded economic development. The developing countries, many of which are in Asia or Africa, typically have low incomes per capita (1). Low incomes lead to low state revenues, limited education, and limited industrial development.

The combined populations of the poor nations are twice that of the wealthy nations and the margin is continually increasing (2). In the United States the population is growing at about 0.8% per year with an expected doubling-time of 70 - 87 years (1, 2). In contrast, Colombia, Morocco, Thailand, and Pakistan have a 3.3% growth rate which can double the population in 21 years. An even faster growth rate is found in Mexico where the population has the potential to double in 15 years (1).

Such statistics show that any advances in improving food yield on this planet will be in vain if they are not correlated with a reduced population growth rate. Food production is restricted by usable land, available water, and man's knowledge and ability to use those resources wisely. There is no available technology capable of producing food at the rate the human population is able to grow. However, the basic research to determine the total agricultural potential of the world has yet to be done. One facet of the problem and one potential solution

is research on wide hybridization and the improved efficiency of cereal crops.

The nutrition problem: quantity versus quality

Improvement of the yield solves only part of the problem. The quality of the food produced can alleviate even more nutritional problems. With the discovery of the opaque-2 gene effect in maize (10), plant breeders could improve the nutritional value of maize by several times (11). The long sought demonstration of genetic control of endosperm nutritional quality stimulated research in numerous cereals. In subsequent years, nutritionally improved cereals for the developing countries has been the target of cereal breeding programs worldwide. These studies have focused on wheat, maize, rice, oats, rye, sorghum, millet, and barley for these are the major staples of the developing countries (5, 6, 8, 12, 13). As cereal protein has been found to be deficient in one or more of the essential amino acids (EAA), diets which are composed primarily of cereal products are nutritionally inadequate (14, 15).

Proteins are critical constituents of the foods consumed by animals and man. They are consumed by animals or man and are hydrolysed in the digestive system into their individual amino acid building blocks or into short polypeptides (16, 17). In these forms they are able to enter the organisms' metabolic pathways where they are used in tissue production and repair, two processes vital to the continuation of life.

Some amino acids can be synthesized within the animal organism but the EAA cannot. Consequently, preformed EAA must be supplied in the diet in sufficient amounts to meet the needs of each individual.

The EAA required can vary between animal species although eight of them are commonly EAA for animals. The human requires those eight amino acids (18) which are:

Valine	Methionine
Leucine	Threonine
Isoleucine	Phenylalanine
Lysine	Tryptophan

Studies with the growing infant human have shown histidine should also be provided in the diet to promote proper growth (17). The rat also requires those nine amino acids needed by the infant human and, in addition, arginine (19). The rat is able to produce arginine but not in sufficient quantities to promote growth. In contrast, growing chicks require 13 amino acids within their diet (20).

Proteins from different food sources have different amino acid profiles. That is, different proportions of each amino acid are specific for each protein. Those proteins which supply the proper balance of essential and nonessential amino acids, approximately 33% and 66% respectively (21), are called complete proteins and have a high biological value. These generalizations suggest that sufficient quantities of each amino acid are present to promote the growth and repair of tissue of growing or adult individuals. Poor quality proteins have lower proportions of EAA and cannot readily promote the growth or repair of tissue required to maintain the organism. Consequently, the organism will slowly deteriorate from attempting to maintain essential services and die.

Meat and milk products have proteins of high biological value, but plant proteins are generally poorer in protein quality (15). The wealthy nations, largely the western-world countries, consume a mixed

diet of cereals, legumes, and meats (1). This mixed diet balances poor quality protein with high quality protein. Meat products are high in cost, however, and are less efficiently produced than plant products in terms of energy consumed. To produce meat products, animals must consume large amounts of plant material (potential human food) in order to produce the animal meat and milk substances (9, 15). The end product, however, has a higher biological value than the initial plant material.

The developing countries cannot afford to convert valuable plant crops into animal tissue nor can they afford to purchase animal products from the wealthier nations. Tradition and religious restrictions may also prohibit the consumption of certain animal products (1, 22). Consequently, the developing countries will continue to rely upon cereal crops as their primary food sources.

Potential solutions to food problems

There are three basic approaches to the problem of improving food resources in underdeveloped countries. One is to introduce new sources of food, although new foods are not easily accepted and may be rejected due to religious or traditional mores (22, 23). The question of finding new food crops which can be economically produced within the country is another problem. A second solution is to fortify cereal foods with EAA to improve the nutritional quality (9, 24). The expense and the task of reaching the rural populations, particularly through an infinite number of cereal processing units, would make this solution impractical for more than half of the world's population. Furthermore, the diet may be too poor nutritionally or may require large quantities of water for cooking which would leach out the added EAA, as in the case

of cassava. Thus, supplementation is impractical in many cases (9). The third solution is to improve present crops genetically to increase the quality and quantity of the protein produced. This approach appears most practical for all situations, although it is time consuming and expensive.

Evolution of crossability barriers

Cereals were domesticated about 10,000 years ago (25). The theory of evolution states that what we now characterize as cereal species developed through millions of years of evolution and selection from a single life form (26). Studies have supported the theory of a common origin among the cereals (27, 28). Evolution is a logical supposition, because comparisons of proteins between cereal species by means of electrophoresis, amino acid composition, and amino acid sequencing show common qualities which are not likely due to chance.

One aspect of evolution is the development of separate and distinct breeding populations with unique gene pools. Through the intervention of geographical, physical, or biochemical barriers the diverging populations within the animal and plant kingdom became unable to interbreed. These crossability barriers maintained cross breeding within populations but prevented crosses with other populations. In the past, as today, man has been able to control the geographical and physical barriers, but the biochemical or molecularly mediated barriers have been largely insurmountable.

What are the barriers that remain after the geographical and physical barriers are passed? A concept has been developed which conceives of the incompatibility as immunochemical reactions similar to those produced in animal tissue grafts--reactions in which foreign

tissues are rejected. The plant kingdom has been believed exempt from such responses although models have been proposed for self-incompatibility based on immunochemical concepts (29). For cross-incompatibility, the concept of stereospecific inhibition reactions (SIR) has been suggested to account for the barrier action until the incompatibility can be further defined (29).

Wide-hybrid crossing

Attempts have been made at wide-hybrid crossing for many years but few successes have been reported (30, 31, 32, 33, 34). Some plant breeders believe that if more distantly related populations could be crossed, it would provide a means of expanding the gene pool of the progeny of that cross. Expanding the gene pool by incorporating the genes from other breeding populations could produce new and greater variations in yield, disease resistance, and protein quality.

Triticale originated when the F_1 hybrids of rye x wheat were treated with colchicine to double the chromosomes and produce a fertile plant (35). It was desirable to cross wheat and rye to combine the qualities of the two species. Rye, largely used in animal feed, has good quality protein and is able to grow in conditions that are too harsh for wheat (36). Wheat, the most extensively grown cereal crop, is the only cereal having the protein quality applicable to leavened bread. However, the protein of wheat is limited in the essential amino acid, lysine (12). The combination, triticale, can be grown in cooler and higher elevations than can wheat (35) and the nutritional value is superior to wheat (37, 38).

If intergeneric crossing can be achieved between other cereal species, it would open new freedoms in plant breeding. A barley x rye

cross is of interest because barley has a recessive gene which increases lysine and other amino acids, regardless of the protein content (38). Furthermore, barley and rye are nearly identical in amino acid composition with a lysine, methionine, and threonine content greater than triticale. Consequently, since triticale has a protein quality and quantity greater than wheat in human feeding studies (39) and equal to rye in rat studies (40), a successful cross between barley and rye could produce a cereal with protein superior to triticale. The lysine gene in barley may also produce a protein superior in quality to either barley or rye when incorporated with the rye gene complement. In a wheat x barley cross it is likewise desirable to transfer the lysine gene into wheat.

Research efforts toward intergeneric hybridization in the Kansas State University-CIMMYT cooperative project have met with limited success. Successful crosses have been reported between wheat x barley and barley x rye (7, 41). The techniques used include crosses based on natural crossability and those crossability barrier(s) which occur(s) in plants and may be similar to animal immunochemical systems. Chloramphenicol, ε-aminocaproic acid, gentisic acid, salicylic acid, and acriflavin have been used in hopes of suppressing the chemical barriers to crossing.

Hybrids are generated in the following manner (7): The chemical or distilled water is injected into a leaf spike of the "maternal" plant prior to egg cell meiosis and continued periodically through emasculation. Untreated pollen is then applied to the floret. The embryos are excised at 20 days and those that germinate are grown to seedlings in a culture medium. Seedlings are transferred to peat pots, root tip samples are taken to make cytological studies on chromosome

behavior, and the seedling is then taken to the green house. Colchicine is applied to selected tillers to induce chromosome doubling to hopefully produce a fertile plant. Grain from fertile plants exhibiting interesting or desirable characteristics is harvested for quality studies. The methodology for kernel structure evaluation and the results of protein analysis are presented in this thesis.

PART I

A DIMETHYL SULFOXIDE PREPARATIVE TREATMENT FOR SECTIONING CEREAL GRAINS

Kernel structure has been important in characterizing cereal species and in correlating physical variations with milling and other processing properties (35, 42, 43, 44, 45, 46, 47, 48). Pericarp and endosperm structures can be used as guides to flour yield and, in the case of hybrids, may display characteristics inherited by the progeny from intergeneric hybridization experiments.

The structures can provide morphological markers, unique physical attributes, which can demonstrate inheritance of that trait from parents to offspring. Maize has a morphological marker in the density of the grain endosperm (49). Barley has a similar marker in its multiple aleurone layer of the kernel (50). Since wheat and rye possess a single aleurone layer, a cross with barley as the paternal parent that produced a multiple aleurone offspring would imply gene transfer in a successful wide-hybrid cross. Also of interest would be the development of abnormal structures not consistent with either parent. This would indicate new gene interaction and exchange of genetic material.

Due to the dry and brittle nature of cereal grains, sectioning of the kernels is impossible without pretreatment to soften the tissue. Conventional methods of paraffin or resin embedding followed by cutting with glass or factory machined knives are laborious, time consuming, and often expensive (44, 51, 52).

It was desirable to develop a single technique which softened the kernel without altering its structure by physical disruption or enzyme activation. It is very difficult for liquids to penetrate through the

cutinized surface of the pericarp and the dense endosperm of mature kernels (51). Dimethyl sulfoxide (DMSO) was chosen as the softening medium because it penetrates tissue rapidly with little or no damage to the tissue (53, 54). For these reasons it is often used as a carrier of pesticides, dyes, and nutrients. DMSO also inhibits enzymatic activity (55, 56, 57, 58), respiration, nucleic acid synthesis, and other cellular processes (59, 60). Consequently, DMSO can limit artifact development due to germination, a common occurrence when the softening medium contains water. Furthermore, DMSO has a protective action on protein (61). This makes it a good extraction medium for proteins prepared for electrophoresis (62). These qualities permitted development of a rapid sectioning method for wheat, barley, rye, and their intergeneric hybrids for the routine evaluation of structural changes.

MATERIALS AND METHODS

Cork mounts were prepared from #1 cork stoppers by removing approximately 5 mm. from the narrow end. The remainder was punctured to permit pressing and receiving of a kernel (germ first) into the cork. The end of the cork penetrated by the kernel was then secured to a wooden dowel 8 mm. in diameter by 4 cm. long with a fast setting epoxy adhesive. The kernel was positioned near the surface of the cork so that the epoxy bond would also provide direct support to the kernel as well as the cork. The brush end provided a stronger bond than did the germ structure.

After the epoxy had set (30 min.), the mounts were cut back to expose the endosperm of the kernel and to permit more rapid DMSO penetration. The mounts were then inverted and sealed in 6-dram screw cap vials into which had been added five drops of 80% (V/V) aqueous DMSO.

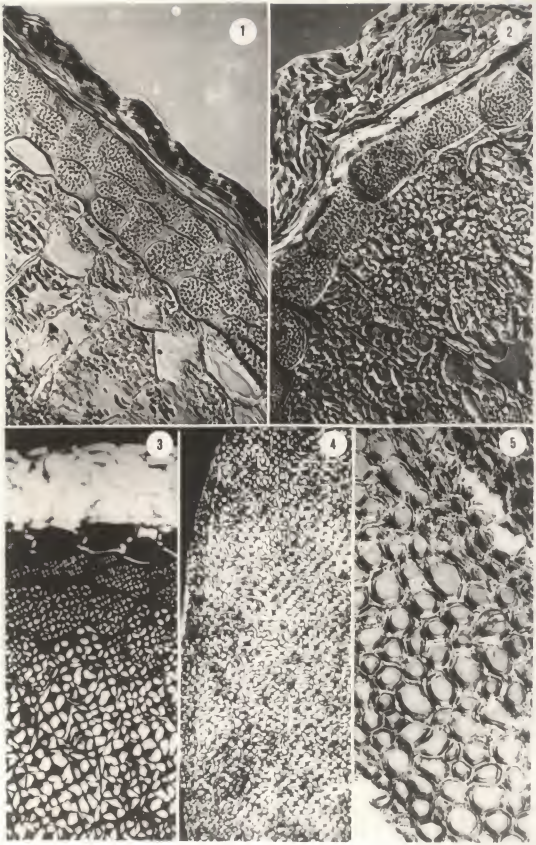
Excess solution was found to soften the epoxy.

The mounts were removed from the solution after four days and air dried four additional days. Each preparation was sectioned with a rotary microtome with a razor blade adapter. The sections were cleared and washed in methanol, xylene, and clove oil for 15, 10, and 30 minutes, respectively, and mounted in a synthetic resin. Interfering debris from sectioning the cork mount was removed during washing and clearing. Once mounted the sections were studied and photographed through phase contrast, polarized light, and Nomarski interference-contrast microscopy.

RESULTS AND DISCUSSION

The cork mount provides two important functions in this procedure. Uniform penetration of the DMSO solution appears to be controlled by the cork. When not embedded in cork, kernels become very soft and distorted in shape, but within cork the kernels maintain their shape during pretreatment and drying. The cork is sufficiently rigid to support a soft kernel while easily sheared by the microtome. Some lateral movement does occur because of its soft nature and the further softening action of DMSO (63). This can cause some variation in section thickness. That problem may be alleviated by using a larger dowel with more mounting base surface for the cork and by changing razor blades more often.

Plates 1, 2, 3, 4, and 5 display photographs of sections prepared by this procedure. Sections of 12 to 15 μm. thick can be prepared readily. Thinner sections of 6 to 10 μm. (Plate 1) are more difficult and exhibit more variation in thickness with disruption of cellular contents. The additional thickness is not detrimental because Nomarski


1
2
3
4
5

interference-contrast technique permit optical sectioning of cleared kernel sections (Plates 1, 2, and 5). The Nomarski technique also eliminates the haloed edges around preparations which are encountered often in phase contrast and gives a three dimensional perception of details which can be enhanced with various wavelengths of light and with different stage orientations (64, 65).

Solubility studies of starch in DMSO have conducted (66, 67). With high ratios of DMSO to starch, some starch granules are solubilized while others continue to display birefringence. Observations of the DMSO prepared sections clearly display pericarp layers, aleurone cells, endosperm cells, and birefringent starch granules (Plates 3 and 4) with no apparent artifacts. The action of DMSO in this procedure is not clear, but it may substitute for the bound and free water (57, 58) in the kernel. As the kernel is dried, the DMSO, being non-volatile, remains within the kernel making it soft.

CONCLUSIONS

This sectioning technique was developed in hopes of identifying inherited or abnormal structures in the wide-hybrid offspring produced at Kansas State University. Approximately 150 hybrid kernels were sectioned with this technique but none showed any detectable inherited paternal traits or the occurrence of abnormalities in starch granules, endosperm cells, or aleurone. Despite these results the technique adds a fast and simple means of observing kernel structure. Although the initial pretreatment with drying period is long, the small amount of labor involved and lack of visible artifacts warrent its consideration over conventional sectioning methods for routine kernel structure observations. The technique was found applicable to other cereals,

legumes, and oilseeds. Because DMSO is a reversible inhibitor of enzymes (56), this technique offers a simple method to study the intracellular location of enzymes through vital and functional staining.

PART II

PROTEIN ANALYSIS OF POTENTIAL WIDE-HYBRIDS

Protein analysis of cereals encompasses numerous techniques which are used to judge nutritional quality and to study the biochemical "markers". Typically studied are the total protein content, the fractional composition of the proteins, and amino acid composition of the whole grain or of the protein fractions. The importance of nutritional protein quality has been explained. Part II examines the application of protein electrophoresis of gene markers to the qualitative inheritance of exotic genes in wide-hybrid breeding experiments.

Physical characteristics or phenotypic markers can greatly aid breeding research by eliminating chemical analysis (49). Opaque-2 and floury-2 maize display markers in the density and softness of their endosperm and the opacity of the kernels to light. In most cases where modifier genes do not reverse the phenotype, the opaque-2 effect can be followed directly as a correlated marker of protein quality. Although other cereals show seed shriveling, shrunken grain traits, and endosperm texture differences, these have not been linked to protein quality genes (12, 49, 68, 69) but, instead, to defective endosperm development. Consequently, the chemical analysis of protein remains a primary method of studying inheritance in progeny.

Because amino acid and protein synthesis control is not well understood in terms of identified genes, one must seek variations in protein composition which are outside the normal expected range of either parent. In the electrophoretic patterns of proteins, we are looking for

the addition or deletion of bands relative to the parental protein banding patterns. Additionally, bands which have the same relative mobility and enzymatic function in parents as well as progeny can be used as reasonably accurate evidence of identical gene action. If quantified amounts of proteins are applied to the electrophoresis gels, one man also measure variations in band density as a measure of gene action.

Changes in amino acid profiles were found in the development of opaque-2 maize (Table 1) and triticale (Table 2). Opaque-2, when compared with the amino acid composition of normal maize, showed a significant increase in lysine accompanied by a marked change in many of the other amino acids. Yong and Unrau (70) analyzed the amino acid profiles of the protein fractions of triticale and its parents and found that a decrease in an amino acid of one fraction of triticale protein was compensated by an increase in that amino acid of another fraction. When floury-2, another maize mutant, was analyzed and its amino acid composition compared to opaque-2, methionine was found to be much greater while lysine was one-third that of the opaque-2 (71). Furthermore, unpublished data by J. R. Jimenez T. (71) indicate zein proteins from opaque-2, floury-2, and normal maize produce different banding patterns.

Electrophoresis is a valuable tool for providing "finger prints" of genotypic expression. Ellis (72) found that he could distinguish between genotypes of wheat, rye, and the hybrid triticale from the electrophoretic patterns of the albumins, globulins, and gliadins. Chen and Bushuk (73 - 75) studied total protein, amino acid composition, protein solubility fractions plus the molecular weights and electrophoretic patterns of the fractionated albumins, globulins,

TABLE 1. AMINO ACID COMPOSITION OF OPAQUE-2 AND NORMAL MAIZE[a]

	Normal Maize	Opaque-2 Maize
	%	%
Lysine	2.00	3.39
Histidine	2.82	3.35
Ammonia	3.28	3.41
Arginine	3.76	5.10
Aspartic Acid	6.17	8.45
Threonine	3.48	3.91
Serine	5.17	4.99
Glutamic Acid	21.30	19.13
Proline	9.67	9.36
Glycine	3.24	4.02
Alanine	8.13	6.99
Cystine	1.79	2.35
Valine	4.68	4.98
Methionine	2.83	2.00
Isoleucine	3.82	3.91
Leucine	14.29	11.63
Tyrosine	5.26	4.71
Phenylalanine	5.29	4.96

[a] Mertz, E. T., Bates, L. S., Nelson, O. E. Science 145:279 (1964).

TABLE 2. AMINO ACID COMPOSITION OF TRITICALE AND ITS PARENTAL FLOURS[a,b]

	Spring Rye (CV. Prolific)	Triticale (CV. 6A190)	Durum Wheat (CV. Stewart)
Lysine	3.49	2.80	2.29
Histidine	2.14	2.34	2.37
Ammonia	3.40	3.25	3.91
Arginine	4.55	4.77	3.64
Aspartic Acid	6.82	5.67	4.62
Threonine	3.26	3.05	2.82
Serine	4.11	4.37	4.37
Glutamic Acid	30.51	32.91	35.78
Proline	15.29	14.18	13.92
Glycine	3.82	3.87	3.52
Alanine	4.06	3.55	3.27
Cystine	2.65	3.22	2.66
Valine	5.22	4.93	4.77
Methionine	2.15	2.25	2.14
Isoleucine	4.21	4.37	4.51
Leucine	6.65	7.55	7.46
Tyrosine	2.16	2.81	2.67
Phenylalanine	5.16	4.98	5.48

[a] Bushuk, W. Proteins of Triticale: Chemical and physical characteristics. In: "Triticale: First man-made cereal". Tsen, C. C., American Association of Cereal Chemists, Inc., St. Paul, Minn. p. 135 (1974).

[b] Grams amino acid per 100 gram protein (N x 5.7). Tryptophan not determined.

prolamines, and glutelins of triticale and its parents. They did not find any bands which were not present in either parent; that contradicted the earlier findings of Yong and Unrau (76) who had discovered four new hybrid bands in triticale not present in either parent. The discovery of hybrid triticale bands remains unresolved at present.

Doekes (77) checked 80 varieties of wheat and believed it was possible to identify the genomes by densitograms of the electrophoretic patterns of the albumins and prolamines. Ewart (27) and Booth and Ewart (28) applied immunological tests to the electrophoretic patterns of cereals to test the theory of a common origin. Tests of eight varieties of wheat flour and a rye flour showed five antigens were common. Barley indicated only three antigens were common, while oats and maize did not react significantly. These data, when coupled with amino acid profiles and amino acid sequences of protein fractions, would imply a common origin among wheat, rye, and barley rather than the chance happening of these common characteristics (28). This is the foundation for attempting wide-hybridization of these genera and the transfer of advantageous genes for nutritional quality improvement.

MATERIALS AND METHODS

Sample preparation

All cereal samples were ground and defatted prior to protein analysis. A "WIG-L-BUG" Amalgamator (Crescent Dental Mfg. Co., Chicago, IL) was used to pulverize the kernels to a flour consistency. Care was taken not to overheat the material during grinding. The procedure was time consuming, as only a few kernels could be ground at one time; however, it allowed efficient recovery of small amounts of material as

opposed to the larger milling units available. This was important as, similar to F_2 cereal breeding experiments, there were only a few grams of material available to sacrifice for analysis.

The ground material was defatted with hexane in a Soxhlet apparatus for 12 hours, the solvent evaporated, and the material sealed in vials and stored at room temperature. Protein (Kjeldahl N x 5.7), amino acid analyses, and electrophoresis were conducted on an "as is" moisture basis, with weight measurements made to four decimal places.

Total nitrogen

Nitrogen was determined by the AOAC micro Kjeldahl method (78). The factor 5.7 (except where indicated) was the extrapolation factor used for converting nitrogen to protein according to the research done by Tkachuk (79 - 82). Each sample was digested in a 30 ml. micro Kjeldahl flask on an electrically heated digestion rack. Ammonia was distilled via a LABCONCO micro-distillation apparatus into a 125 ml. Erlenmeyer flask and titrated with 0.1N HCl standardized by the AOAC standard borax method (78).

Amino acid determination

Hydrolysis: A modified procedure of Liu and Chang (83) using p-toluenesulfonic acid (p-TSA) as the hydrolysing agent was used. A sample equal to 10 to 15 mg. of protein was placed in a 16 x 150 mm. culture tube with 10 ml. of 3N p-TSA and 10 micromoles of norleucine internal standard (84).

The tubes covered with polypropylene caps (Sherwood Bacti Capalls) and placed in an enclosed boiling water bath for 31 hours. The tubes were removed and allowed to cool several minutes, and 4 ml. of 6N NaOH in pH 2.2 sodium citrate buffer was added. The citrate buffer was

prepared according to Moore et al. (85). The hydrolysate was transferred to a 25 ml. volumetric flask and diluted to volume with pH 2.2 sodium citrate buffer. Each hydrolysate was filtered through a 0.2 μm. pore size membrane filter using a syringe equipped with a swinney filter adapter (Becton, Dickinson and Company, Rutherford, NJ). Hydrolysates were stored in sealed vials at -20°C.

Amino acid analysis: The analyzer was standardized with an "in-lab preparation" of the 17 amino acids, ammonium chloride, and norleucine. The analysis followed a 2-hour methodology according to Hubbard (86) using a Beckman 120C amino acid analyzer equipped with a normal 5-millivolt range card.

Electrophoresis

Approximately 0.4 grams of ground, defatted sample were mixed with 3 ml. of DMSO (30% aqueous) in a 9 ml. vial. The vials were positioned horizontally on a homemade rocker/shaker producing forty 360° rolls of the vials per minute. Extraction continued for 24 hours at room temperature. The mixture was centrifuged at 10,500 rpm for 15 minutes and the supernate stored at -20°C.

Electrophoresis of the protein extracts followed the procedure of Davis (87) using a polyacrylamide matrix (Table 3). Acrylamide and bisacrylamide were recrystallized according to Loening (88). A Hoefer twelve-capacity disc gel chamber, cooled by a Haake FK2 constant temperature circulating water bath, and powered by a Gelman constant voltage source was used. The gels were compsoed of a 7% acrylamide separation gel (9.5 cm. in height) topped with a 1 cm. stacking gel of 2.5% bisacrylamide.

The DMSO-protein extract was applied to the top of the stacking

TABLE 3. ELECTROPHORETIC REAGENTS[a]

Ammonium Persulfate (AP)

14 mg. per 10 ml. DDW[b]

Electrode Buffer (upper & lower)

12 g. Tris Base
57.6 g. Glycine
Dilute to 1 liter via DDW
pH 8.3
(Final dilution 1:10)

Solution A

46 ml. HCl
36.3 g. Tris Base
0.46 ml. TEMED
Dilute to 100 ml. via DDW
pH 8.9

Solution B

1N HCl (48 ml.)
Tris Base (5.93 g.)
TEMED (0.46 ml.)
Dilute to 100 ml. via DDW
pH 6.7

Solution F

40 g. Sucrose
Dilute to 100 ml. via DDW

Solution C

45 g. Recrystallized Acrylamide
1.2 g. Recrystallized Bisacrylamide
Dilute to 100 ml. via DDW

Solution D

10 g. Recrystallized Acrylamide
2.5 g. Recrystallized Bisacrylamide
Dilute to 100 ml. via DDW

Solution E

4 g. Riboflavin
Dilute to 100 ml. via DDW

Upper Stacking Gel Ratios

B : D : E : F
1 : 2 : 1 : 4

Lower Stacking Gel Ratios

A : C : DDW : AP
4 : 6 : 6 : 16

[a] Davis, B. J., Ann. N. Y. Acad. Sci. 121: 404 (1964).

[b] Double distilled water.

gel and current was applied at the rate of 1 mA per tube. Bromophenol blue was included in the upper buffer reservoir to provide the tracking dye front. When the tracking dye had traveled about 0.5 cm. into the separation gel, power was increased to 2 mA per tube until 30 minutes past the time the tracking dye left the bottom of the separation gel. Because the tracking dye did not migrate uniformly through all the gels, the 30 minute times were individually noted. Although the chamber was equipped with a horizontal balance mechanism, it is suspected that there is an error in construction such that the chamber cannot be balanced using the horizontal balance guide.

RESULTS AND DISCUSSION

p-TSA is advantageous over the commonly used hydrochloric acid hydrolysis because it does not have to be removed from the hydrolysate prior to injection into the analyzer (83, 89). Although not a factor in the present study, p-TSA allows the determination of tryptophan. It is a milder acid and, with reduced amounts of carbohydrates, it is not as destructive toward the more easily oxidized amino acids (83).

All digestions included norleucine as an internal standard. Norleucine is an analog of leucine and does not occur naturally. When the sample is injected into the amino acid analyzer the peak for norleucine occurs several minutes after the peak for leucine. The internal standard allows checks to be made on the efficiency of recovery and technique by correcting for mechanical losses, although it does not correct for hydrolytic losses.

Dimethyl sulfoxide (DMSO) was used to extract the proteins from the cereal samples. The procedure was based on the research done by Ascher and Weinheimer (62) which showed that DMSO was comparable to

other extraction techniques used for electrophoresis. The extraction can be conducted at room temperature without noticeable denaturing of protein. This eliminates the need for cooling apparatus. Furthermore, the warmer extraction should allow a more efficient recovery of protein. The procedure was modified to be applicable to the small amount of sample available and the rocker system used for agitating the mixture.

Data from the analysis of potential hybrids and parental types are presented in Figures 1 and 2 and in Tables 4 and 5. Figures 1 and 2 present the protein electrophoretic ideograms while data related to total protein and amino acid composition are presented in the Appendix Tables.

Samples in Figure 1 are described in Appendix Table 1 (page 40) where E, F, H, and I are the potential hybrids from crosses of Cocorit 71 (Durum wheat) x Apizaco (barley). Likewise, K and L originated from crosses of Cocorit 71 x Promesa (barley).

Ideally, there are three characteristics of hybrid banding patterns which would indicate successful transfer of genetic material from the pollen parent (barley) into the progeny. 1) The existence of bands not common to either parent; 2) the discovery of bands common to the paternal parent but not the maternal parent; and 3) the elimination of bands common to the maternal parent.

It is possible that gene function, in this case the production of proteins, may be turned off or on by the transfer of genetic material (a fragment of DNA) through an insertion principle that has been observed in bacteria and viruses (90, 91). The occurrences are explained in the theory proposed by Bates et al. (92, 93). Such a transfer of DNA in fragments is conceivable when there is much homology between the maternal and paternal genome. Some sequence similarity has been detected

Figure 1. Protein electrophoretic patterns of potential wide-hybrids and parental types described in Appendix Table 1. Material was not available for the analysis of samples A and B.

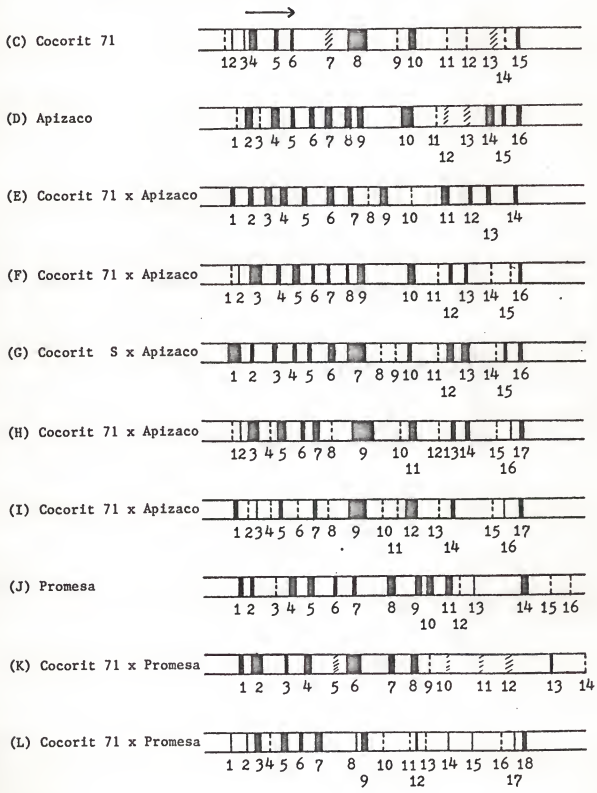


Table 4. Comparisons of the electrophoretic patterns of the potential wide-hybrids with Cocorit 71 (maternal parent) as presented in Figure 1 and Appendix Table 1.

Sample #	Added bands[a,d]	Deleted bands[b,d]	Common bands[c]
E	(3), (4), (5), (9), 14	(1), (3), (5), (6), (9), 13, (14)	1, 2, 6, 7, 8, 10, 11, 12
F	1, 2, (6), 8, (11), 15	1, 2, (3), (9), 14	3, 4, 5, 7, 9, 10, 12, 13, 14, 16
G	3, (5), (8), 9, 10, (11), 15	(3), 5, 9, 10, 14	1, 2, 4, 6, 7, 12, 13, 14, 16
H	1, 2, (4), 6, (7), (12), 14, 16	1, 2, 6, 12, 14	3, 5, 8, 9, 12, 14, 17
I	(4), (7), 8, (10), 11, (13), 15, 16	(1), 7, 9, (12), 13, 14	1, 2, 3, 5, 6, 9, 12, 14, 17
K	1, 3, 4, 7, 9, 10, 11, (13), (14)	(1), 2, 3, 5, 6, 9, 11, 12, 13, 15	2, 5, 6, 8, 12
L	2, (4), (7), (10), 11, 13, 14, 17	(2), 3, (7), 9, 11, 14	1, 3, 5, 6, 8, 9, 12, 15, 16, 18

NOTE: Samples E, F, H, and I are hybrids from a Cocorit 71 x Apizaco cross. Sample G is also compared with Cocorit 71 although it is actually a cross of a Cocorit 71 (sibling) x Apizaco. Samples K and L are hybrids from a Cocorit 71 x Promesa cross.

[a]Added bands are the bands in the sample which do not match the position of any bands in the maternal.

[b]Deleted bands are the bands appearing in the maternal which do not match any bands in the sample.

[c]Common bands are bands of the sample which match the band positions of the maternal.

[d]Parentheses around a band number indicate that band is unique and, although there was unequal tracking dye movement, the band cannot match the position of any band or bands it is compared with.

Figure 2. Protein electrophoretic patterns of potential wide-hybrids, parental types, and maternal control treatments described in Appendix Table 8.

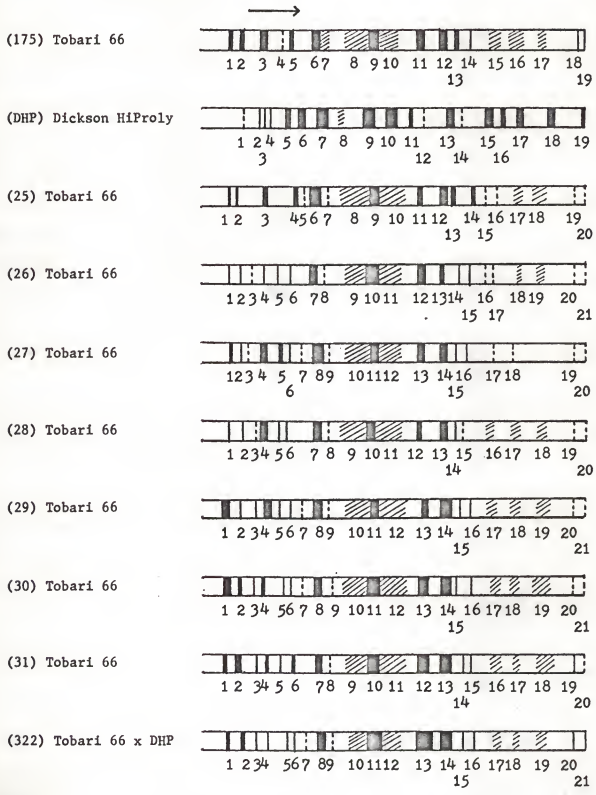


Table 5. Comparisons of the electrophoretic patterns of the maternal controls and potential wide-hybrids with Tobari 66 (maternal parent) as presented in Figure 2 and Appendix Table 8.

Sample #	Added bands[a,d]	Deleted bands[b,d]	Common bands[c]
25	2, (5), 15, 19	2, (4), 8	1, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 20
26	1, 2, (3), 5, 15, 16, 20	1, 2, 4, 18	4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 17, 18, 19, 20
27	3, 5, 6, (7), 16	4, 5, 14, (17), 18	1, 2, 4, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 20
28	1, (3), 5, 6, 15, 19	4, 5, 14, 18	2, 4, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 20
29	(3), 5, (7), 15, 20	4, 13, 18	1, 2, 4, 6, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 21
30	(3), (7), 9, 20	18	1, 2, 4, 5, 6, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21
31	1, 2, (3), 5, 14, 19	1, 2, 4, 13, 18	4, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 20
322	1, (3), 5, (7), 9, 15, 16, 20	1, 4, 13, 14, 18	2, 4, 6, 8, 10, 11, 12, 13, 14, 17, 18, 19, 21

NOTE: Samples 25 - 31 are control treatments of the maternal parent. Samples 25 - 28 were treated with EACA and samples 29 - 31 were not treated with EACA. Sample 322 was found identical to samples 322 - 362 and is used to represent the potential hybrids generated in the Tobari 66 x Dickson HiProly cross.

[a]Added bands are the bands in the sample which do not match the position of any bands in the Tobari 66.

[b]Deleted bands are the bands appearing in the Tobari 66 which do not match any band positions in the sample.

[c]Common bands are bands of the sample which match the band positions of the Tobari 66.

[d]Parentheses around a band number indicate that band is unique and, although there was unequal tracking dye movement, the band cannot match the position of any band or bands it is compared with.

between wheat, rye, and barley (94).

The chemical treatment is an attempt to prevent synthesis of SIR precursors, beginning at egg cell meiosis, without detriment to pollen function (95). Because of homology, parts of the maternal and paternal chromosomes may be competing for attachment sites on the nuclear membrane thus disturbing replication. Then, subsequent to excision and repair by enzymatic activity (96), fragmentation with insertion of the paternal DNA into the maternal chromosome is theorized. This is followed by the elimination of the remaining DNA (97, 98), as our studies show paternal chromosomes are lost early in embryogenesis (92, 93). The "maternal" genome is then replicated along with the newly inserted fragment. Consequently, protein synthesis is changed producing proteins with altered amino acid sequences, molecular weight, shape, or charge. This would then change the profile of the proteins in electrophoresis.

Figures 1 and 2 represent enlarged ideograms of the original gels which were 9.5 cm. long. Furthermore, because the tracking dye did not move uniformly, the gel banding patterns were aligned with each other according to groups of corresponding bands. Care should be taken in judging band relationships within Figures 1 and 2, because the tracking dye did not move uniformly. This would have been irrelevant when using the R_f method of plotting band movement because band positions are relative to the movement of a control substance. But in this study, the R_f measurement cannot be made as the current was continued 30 minutes after the tracking dye front reached the ends of the gels. The current was continued to improve band separation and in hopes of separating some bands which would otherwise appear as one band (99).

In the absence of R_f measurements, the transposed ideograms in Figures 1 and 2 and the analysis of band relationships in Tables 4 and

5 do show considerable alignment between the common bands. However, there are a considerable number of bands present in the maternal parent not present in the progeny. Furthermore, there are a similar number of bands observed in the progeny that were not detected in the maternal parent.

Considering the problem with tracking dye movement and the fact that current was continued 30 minutes after the tracking dye left the gels, the band numbers in parentheses are the most accurate interpretation of new or deleted bands. These bands are not believed to be displaced bands or bands coming out of separation from other bands. This is a judgment which can be made when considering band groupings in the vicinity of the band in question.

Ideograms in Figure 2 were similarly transposed from the original gels. Table 5 shows a greater proportion of bands listed under common bands than were found in Figure 1 and presented in Table 4. As in Table 4, the band numbers in parentheses represent the bands which are most likely unique. Although there are only two such bands that were deleted (samples 25 and 27), one to two unique bands were added in all samples. It is important to note that the added bands 3 and 7 repeatedly occur in samples 26 through 322. It should also be noted that band numbers do not always correspond from gel to gel; however, in most cases they do correspond in Figure 2.

As explained earlier, the addition or deletion of protein bands would indicate an altered genetic makeup, whereby DNA has shut off the transcription of a protein or proteins or is transcribing the production of a new protein or proteins. In Figures 1 and 2, the addition and deletion of unique bands, indicated by the parentheses, are the most likely indications of altered protein production in the hybrids and,

consequently, the transfer of genetic material from the paternal parent.

Data on protein and amino acid composition may also be an indication of variation between progeny and parents. Appendix Table 1 (page 40) describes interspecific crosses between Tobari x rye, Cocorit 71 x Apizaco, and Cocorit x Promesa. Appendix Table 8 (page 49) describes a similar cross between Tobari 66 x Dickson HiProly. The corresponding amino acid data, total protein, nitrogen recovery, and the related statistics are found following these tables. The amino acid data is presented as per cent of sample material and as per cent of the Kjeldahl protein corrected to 100% recovery of Kjeldahl protein.

In the Tobari x rye cross there were only the maternal parent and one potential hybrid sample to analyze. Total protein is greater in the potential hybrid and is accompanied by a slight increase in lysine when presented either as per cent of samples or per cent of Kjeldahl protein. All other parameters remain similar. Variation does not appear to be too different from the maternal parent.

In the wheat x barley crosses of Appendix Table 1 (page 40), t tests were used to test the mean of the potential hybrids against the maternal control constants. Data for each of the 17 amino acids, ammonia, amino acid protein, Kjeldahl protein, and nitrogen recovery were tested. Looked for were a prominent number of rejections of the hypotheses for the amino acids and/or the protein content. Although a few hypotheses are rejected there does not appear sufficient variation between the means and the control values for the amino acids.

In the Tobari 66 x Dickson HiProly cross and related maternal control treatments, statistical comparisons should be treated lightly because of the disparity of sample sizes. The t tests were done but, to properly be considered, samples are assumed normal with equal variances (100). This

may not be a proper assumption here.

A brief look at the means presented for the two maternal treatments and the potential hybrids (Appendix Table 11 and 12; pages 60 and 61) does not show any drastic differences in either protein or amino acid data. A t test for the equality of two means is presented in Appendix Table 13 (page 62). Here the sample means for the maternal control treated with EACA are tested against the sample means for the maternal control treatment without EACA to determine if the EACA application alone alters protein or amino acid profiles. Similarly, the maternal controls with EACA are compared to the potential hybrid samples. This is risky due to the disparity of sample sizes.

Results show a prominent number of rejections of the hypotheses when the means between the two maternal control tests are compared. That is, EACA appears to affect the amount of protein and a large number of the amino acids. However, the effect is nonsignificant when the data is presented as per cent of Kjeldahl protein. This emphasizes the idea that the amount of protein synthesis within the sample is altered but that the composition of the protein itself is not changed.

Among the maternal controls treated with EACA, sample number 27 stands out in Appendix Table 9 (page 50). Most of the amino acids (except cystine) and, especially, the Kjeldahl protein and total amino acid content are above the means calculated in Appendix Table 11 (page 60). Further t tests were made as in Appendix Table 13 (page 62) on Kjeldahl protein and amino acid protein (total amino acid content), excluding sample 27, to see if the comparison between W/EACA and W/O EACA would become non-significant. Results show the comparison is still significant with $t = 7.67$ and $t = 7.92$ for Kjeldahl and amino acid protein, respectively. These values are greater than the test statistic

of t = 2.78. Consequently, although sample 27 may stand out relative to total protein, the EACA treatment still appears to alter gross protein makeup in the maternal controls.

Finally, t tests were conducted to compare the means of the two maternal control treatments and the potential hybrids with the control values of the maternal parent. Results of these tests are presented in Appendix Tables 14 and 15 (pages 63 and 64). In testing the data for per cent of sample, the comparison of the maternal control constants with the maternal control treated with EACA shows significant in all but the test for lysine and alanine. Similar tests on data presented as per cent of Kjeldahl are not significant. This also implies that the EACA application alone does affect protein content of the sample.

Similar results are found in testing the means for the potential hybrids with the maternal controls. In considering the large sample size and the unlikelihood that more than a few of the subsamples are significantly different from the maternal control, it may be more proper to look within the potential hybrid data rather than compare it to control constants. This may be done by taking a confidence interval about each parameter mean and looking at those subsamples having a parameter (such as lysine) with measurements outside the confidence interval.

CONCLUSIONS

Analysis of the elctrophoretic ideograms of the first group of samples (Figure 1 and Table 4) show protein bands which are either added, deleted, or both in all potential hybrids. Consequently, this indicates the alteration of the genetic makeup of the maternal genotype in the Cocorit 71 x Apizaco and Cocorit 71 x Promesa crosses. Ideograms

of the second group of samples (Figure 2 and Table 5) also display banding patterns that are different but more consistent with the maternal Tobari 66. However, there is little discrimination among the two maternal controls and the potential hybrids.

Total protein and amino acid analysis of both sample groups does not indicate any change in the genetic makeup which would alter protein composition. However, the EACA treatment program, as described earlier in this paper, does appear to cause an increase in the gross protein content of the maternal controls and the potential hybrids.

APPENDIX

APPENDIX TABLE 1. IDENTIFICATION OF PARENTS AND F_4 PROGENY.

Code	Field No. BV/74	Lab No. Y-73	Description: History	Chromosomes: Somatic Count	Chromosomes: Pairing
A	6673	687	Tobari x Rye	42	21
B	6900A	-	Tobari (Bread Wheat)		21
C	6620A	-	Cocorit 71 (Durum Wheat)		14
D	6620B	-	Apizaco (Barley)		7
E	6630	225	Cocorit 71 x Apizaco	28	14
F	6650	666	Cocorit 71 x Apizaco	28	14
G	6700	675a	Cocorit S[a] x Apizaco	28	14
H	6745	675b	Cocorit 71 x Apizaco	28	14
I	6810	679	Cocorit 71 x Apizaco	28	14
J	6880B	-	Promesa (Barley)		7
K	6883	691d	Cocorit 71 x Promesa	42	21
L	6888	691g	Cocorit 71 x Promesa	28	14

[a] Cocorit S is a sibling of Cocorit 71.

APPENDIX TABLE 2. PROTEIN CONTENT AND AMINO ACID COMPOSITION OF PARENTAL AND F_4 PROGENY GRAIN IDENTIFIED IN APPENDIX TABLE 1[a]

Sample Code	A	B	C	D	E	F	G	H	I	J
Kjeldahl Protein, %[b]	16.36	15.33	14.99	14.42	15.50	15.73	15.28	15.85	14.93	12.94
Total Amino Acids, %[c]	16.76	15.31	14.86	14.72	15.54	15.73	15.30	16.17	14.96	12.66
Nitrogen recovery[d]	89.83	87.18	86.46	86.20	87.28	87.26	85.46	89.54	87.69	81.99
Lysine	0.45	0.39	0.37	0.43	0.39	0.38	0.39	0.40	0.38	0.37
Histidine	0.39	0.35	0.36	0.30	0.36	0.37	0.36	0.38	0.38	0.26
Ammonia	0.67	0.62	0.62	0.56	0.63	0.65	0.63	0.69	0.62	0.49
Arginine	0.82	0.71	0.66	0.59	0.74	0.72	0.72	0.76	0.69	0.31
Aspartic Acid	1.00	0.88	0.80	0.84	0.89	0.86	0.88	0.91	0.85	0.75
Threonine	0.48	0.43	0.40	0.48	0.41	0.43	0.44	0.43	0.41	0.41
Serine	0.92	0.84	0.84	0.69	0.92	0.85	0.87	0.88	0.77	0.68
Glutamic Acid	5.10	4.76	4.70	4.28	4.84	4.96	4.84	5.11	4.70	3.60
Proline	1.87	1.73	1.72	1.91	1.77	1.81	1.66	1.90	1.73	1.91
Glycine	0.74	0.62	0.55	0.54	0.54	0.61	0.56	0.59	0.58	0.46
Alanine	0.65	0.57	0.52	0.55	0.57	0.52	0.53	0.54	0.53	0.48
Cystine	0.28	0.27	0.22	0.19	0.25	0.26	0.28	0.28	0.24	0.11
Valine	0.51	0.47	0.47	0.51	0.49	0.49	0.48	0.49	0.47	0.45
Methionine	0.22	0.23	0.22	0.21	0.23	0.22	0.20	0.22	0.22	0.18
Isoleucine	0.40	0.38	0.39	0.40	0.40	0.40	0.39	0.41	0.39	0.34
Leucine	1.02	0.94	0.95	0.93	0.98	1.00	0.96	1.01	0.90	0.79
Tyrosine	0.52	0.46	0.41	0.49	0.45	0.46	0.42	0.47	0.43	0.42
Phenylalanine	0.72	0.65	0.67	0.84	0.69	0.72	0.68	0.73	0.68	0.66

[a] Results are reported on an "as is" moisture basis, g. amino acid per 100 g. sample.

[b] N x 5.7

[c] The total weight of amino acids as % of sample.

[d] Nitrogen recovery, g. amino acid nitrogen per 100 g. Kjeldahl nitrogen.

APPENDIX TABLE 2 (concluded).

Sample Code	K	L
Kjeldahl Protein, %	14.65	14.65
Total Amino Acids, %	14.21	14.73
Nitrogen Recovery, %	84.92	87.65
Lysine	0.40	0.38
Histidine	0.32	0.36
Ammonia	0.57	0.59
Arginine	0.70	0.70
Aspartic Acid	0.87	0.81
Threonine	0.38	0.41
Serine	0.74	0.83
Glutamic Acid	4.32	4.56
Proline	1.64	1.66
Glycine	0.60	0.57
Alanine	0.56	0.55
Cystine	0.24	0.24
Valine	0.43	0.47
Methionine	0.21	0.21
Isoleucine	0.34	0.38
Leucine	0.87	0.94
Tyrosine	0.43	0.41
Phenylalanine	0.60	0.65

APPENDIX TABLE 3. PROTEIN CONTENT AND AMINO ACID COMPOSITION OF PARENTAL AND F_4 PROGENY[a] GRAIN PROTEIN WITH AMINO ACID CONTENT CORRECTED TO 100% RECOVERY OF KJELDAHL PROTEIN (N x 5.7)[b]

Sample Code	A	B	C	D	E	F	G	H	I	J
Lysine	2.70	2.56	2.49	2.92	2.52	2.45	2.54	2.47	2.52	2.90
Histidine	2.34	2.29	2.40	2.04	2.33	2.37	2.37	2.36	2.54	2.04
Ammonia	3.99	4.05	4.14	3.78	4.02	4.16	4.14	4.25	4.12	3.83
Arginine	4.91	4.61	4.43	4.00	4.72	4.59	4.71	4.68	4.62	2.43
Aspartic Acid	5.99	5.72	5.38	5.69	5.71	5.48	5.78	5.59	5.69	5.93
Threonine	2.86	2.81	2.72	3.25	2.63	2.74	2.87	2.64	2.73	3.25
Serine	5.48	5.43	5.68	4.66	5.93	5.43	5.71	5.41	5.14	5.39
Glutamic Acid	31.00	30.96	31.58	29.02	31.11	31.60	31.64	31.52	31.40	28.44
Proline	11.18	11.26	11.56	12.95	11.36	11.54	10.83	11.72	11.58	15.07
Glycine	4.41	4.07	3.72	3.65	3.47	3.90	3.64	3.62	3.88	3.61
Alanine	3.87	3.72	3.47	3.76	3.68	3.29	3.50	3.31	3.53	3.76
Cystine	1.64	1.77	1.49	1.30	1.57	1.67	1.85	1.70	1.62	0.87
Valine	3.03	3.07	3.17	3.44	3.11	3.12	3.11	3.01	3.11	3.52
Methionine	1.32	1.48	1.48	1.41	1.45	1.42	1.30	1.36	1.45	1.40
Isoleucine	2.40	2.45	2.61	2.71	2.58	2.54	2.55	2.54	2.57	2.68
Leucine	6.10	6.08	6.40	6.31	6.30	6.34	6.27	6.23	6.02	6.25
Tyrosine	3.10	3.00	2.74	3.30	2.86	2.92	2.76	2.90	2.85	3.28
Phenylalanine	4.27	4.26	4.51	5.70	4.45	4.60	4.43	4.49	4.53	5.24

[a] Identified in Appendix Table 1.

[b] Results are reported on an "as is" moisture basis, g. amino acid per 100 g. protein (N x 5.7).

APPENDIX TABLE 3 (concluded).

Sample Code	K	L
Lysine	2.79	2.61
Histidine	2.26	2.46
Ammonia	3.99	3.97
Arginine	4.89	4.79
Aspartic Acid	6.11	5.53
Threonine	2.68	2.76
Serine	5.18	5.63
Glutamic Acid	30.39	30.98
Proline	11.54	11.31
Glycine	4.21	3.87
Alanine	3.95	3.76
Cystine	1.67	1.60
Valine	3.03	3.17
Methionine	1.46	1.45
Isoleucine	2.42	2.60
Leucine	6.11	6.36
Tyrosine	3.02	2.81
Phenylalanine	4.20	4.45

APPENDIX TABLE 4. MEAN AND STANDARD DEVIATION FOR POTENTIAL HYBRIDS (COCORIT 71 x APIZACO) WITH t TEST TO COMPARE WITH MATERNAL CONTROL CONSTANTS (H_o: $\mu = c$)($t_{0.05,3} = 3.182$)[a] (DATA FROM APPENDIX TABLE 2)

	Control Constant Maternal Parent Cocorit 71 (Sample C)	Cocorit 71 x Apizaco (Samples E, F, H, I)			
		$\bar{X}$	S	t	Accept H_o
Amino Acid Protein	14.99	15.50	0.41	2.49	Yes
Kjeldahl Protein	14.86	15.61	0.51	2.90	Yes
Nitrogen Recovery	86.46	87.94	1.08	2.74	Yes
Lysine	0.37	0.39	0.01	4.00	No
Histidine	0.36	0.37	0.01	2.00	Yes
Ammonia	0.62	0.65	0.03	2.00	Yes
Arginine	0.66	0.73	0.03	4.67	No
Aspartic Acid	0.80	0.88	0.03	5.33	No
Threonine	0.40	0.42	0.01	4.00	No
Serine	0.84	0.86	0.06	0.67	Yes
Glutamic Acid	4.70	4.90	0.17	2.35	Yes
Proline	1.72	1.80	0.07	2.29	Yes
Glycine	0.55	0.58	0.03	2.00	Yes
Alanine	0.52	0.54	0.02	2.00	Yes
Cystine	0.22	0.26	0.02	4.00	No
Valine	0.47	0.49	0.01	4.00	No
Methionine	0.22	0.22	0.01	0.00	Yes
Isoleucine	0.39	0.40	0.01	2.00	Yes
Leucine	0.95	0.97	0.05	0.80	Yes
Tyrosine	0.41	0.45	0.02	4.00	No
Phenylalanine	0.67	0.71	0.02	4.00	No

[a] Zar, J. H. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, N. J. p. 89 (1974).

APPENDIX TABLE 5. MEAN AND STANDARD DEVIATIONS FOR POTENTIAL HYBRIDS (COCORIT 71 x APIZACO) WITH t TEST TO COMPARE WITH MATERNAL CONTROL CONSTANTS (H_o: $\mu = c$)($t_{0.05,3} = 3.182$)[a] (DATA FROM APPENDIX TABLE 3)

	Control Constant Maternal Parent Cocorit 71 (Sample C)	Cocorit 71 x Apizaco (Samples E, F, H, I)			
		$\bar{X}$	S	t	Accept H_o
Lysine	2.49	2.49	0.04	0.00	Yes
Histidine	2.40	2.40	0.09	0.00	Yes
Ammonia	4.14	4.14	0.10	0.00	Yes
Arginine	4.43	4.65	0.06	7.33	No
Aspartic Acid	5.38	5.62	0.11	4.63	No
Threonine	2.72	2.69	0.06	1.00	Yes
Serine	5.68	5.48	0.33	1.21	Yes
Glutamic Acid	31.58	31.41	0.21	1.62	Yes
Proline	11.56	11.55	0.15	0.13	Yes
Glycine	3.72	3.72	0.21	0.00	Yes
Alanine	3.47	3.45	0.19	0.21	Yes
Cystine	1.49	1.64	0.06	5.00	No
Valine	3.17	3.09	0.05	3.20	No
Methionine	1.48	1.42	0.04	3.00	Yes
Isoleucine	2.61	2.56	0.02	5.00	No
Leucine	6.40	6.22	0.14	2.57	Yes
Tyrosine	2.74	2.88	0.03	9.33	No
Phenylalanine	4.51	4.52	0.06	0.33	Yes

[a] Zar, J. H. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ p. 89 (1974).

APPENDIX TABLE 6. MEAN AND STANDARD DEVIATION FOR POTENTIAL HYBRIDS (COCORIT 71 x PROMESA) WITH t TEST TO COMPARE WITH MATERNAL CONTROL CONSTANTS ($H_o: \mu = c$). SAMPLE STATISTICS PRESENTED AS % OF SAMPLE. TEST STATISTIC IS $t_{0.05,1} = {}^{o}12.706$[a] (DATA FROM APPENDIX TABLE 2).

	Control Constant Maternal Parent Cocorit 71 (Sample C)	Cocorit 71 x Promesa (Samples K & L)			
		$\bar{X}$	S	t	Accept H_o
Amino Acid Protein	14.99	14.65	0.00	UD[b]	
Kjeldahl Protein	14.87	14.47	0.35	1.14	Yes
Nitrogen Recovery	86.46	86.29	1.93	0.09	Yes
Lysine	0.37	0.39	0.01	2.00	Yes
Histidine	0.36	0.34	0.03	0.67	Yes
Ammonia	0.62	0.58	0.01	4.00	Yes
Arginine	0.66	0.70	0.00	UD	
Aspartic Acid	0.80	0.84	0.04	1.00	Yes
Threonine	0.40	0.40	0.02	0.00	Yes
Serine	0.84	0.79	0.06	0.83	Yes
Glutamic Acid	4.70	4.44	0.17	1.53	Yes
Proline	1.72	1.65	0.01	7.00	Yes
Glycine	0.55	0.59	0.02	2.00	Yes
Alanine	0.52	0.56	0.01	4.00	Yes
Cystine	0.22	0.20	0.00	UD	
Valine	0.47	0.45	0.03	0.67	Yes
Methionine	0.22	0.21	0.00	UD	
Isoleucine	0.39	0.36	0.03	1.00	Yes
Leucine	0.95	0.91	0.05	0.80	Yes
Tyrosine	0.41	0.42	0.01	1.00	Yes
Phenylalanine	0.67	0.63	0.04	1.00	Yes

[a] Zar, J. H. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, N. J. p. 89 (1974).

[b] Undefined when s = 0.

APPENDIX TABLE 7. MEAN AND STANDARD DEVIATION FOR POTENTIAL HYBRIDS (COCORIT 71 x PROMESA) WITH t TEST TO COMPARE WITH MATERNAL CONTROL CONSTANTS (H_o: $\mu = c$). SAMPLE STATISTICS PRESENTED AS 100% RECOVERY OF KJELDAHL PROTEIN. TEST STATISTIC IS $t_{0.05,1} = 12.706$[a] (DATA FROM APPENDIX TABLE 3)

	Control Constant Maternal Parent Cocorit 71 (Sample C)	Cocorit 71 x Promesa (Samples K & L)			
		$\bar{X}$	S	t	Accept H_o
Lysine	2.49	2.70	0.13	1.62	Yes
Histidine	2.40	2.36	0.14	0.29	Yes
Ammonia	4.14	3.98	0.01	16.00	No
Arginine	4.43	4.84	0.07	5.86	Yes
Aspartic Acid	5.38	5.82	0.41	1.07	Yes
Threonine	2.72	2.72	0.06	0.00	Yes
Serine	5.68	5.41	0.32	0.84	Yes
Glutamic Acid	31.58	30.69	0.42	2.12	Yes
Proline	11.56	11.43	0.16	0.81	Yes
Glycine	3.72	4.04	0.24	1.33	Yes
Alanine	3.47	3.86	0.13	3.00	Yes
Cystine	1.49	1.64	0.05	3.00	Yes
Valine	3.17	3.10	0.10	0.70	Yes
Methionine	1.48	1.46	0.01	2.00	Yes
Isoleucine	2.61	2.51	0.13	0.77	Yes
Leucine	6.40	6.24	0.18	0.89	Yes
Tyrosine	2.74	2.92	0.15	1.20	Yes
Phenylalanine	4.51	4.33	0.18	1.00	Yes

[a] Zar, J. H. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, N. J. p. 89 (1974).

APPENDIX TABLE 8. IDENTIFICATION OF SAMPLES DESCRIBED IN APPENDIX TABLES 9 AND 10.

Sample Code[b]	History			Treatment Schedule[a]	
				W/EACA	W/O EACA
175	Female Parent	Tobari 66 (Bread Wheat)	Control	-	-
DHP	Pollen Parent	Dickson HiProly (Barley)	Control	-	-
25 - 28		Tobari 66 x Tobari 66	Control	Yes	-
29 - 31		Tobari 66 x Tobari 66	Control	-	Yes
322 - 362		Tobari 66 x Dickson HiProly	Potential Hybrids[c]	Yes	-

[a] Includes either application or no application of EACA (injection or spray of -aminocaproic acid on the Tobari 66) followed by emasculation, pollenation, removal of embryo at 15 - 20 days to a culture medium, and growth through generation 4.

[b] Each sample represents seed collected from one plant. Collective rows of plants include 322 - 326, 327 - 336, 337 - 347, 348 - 354, and 355 - 362.

[c] Cytogenetically stable with 21 pairs of chromosomes at generation 2. No Dickson HiProly chromosomes detectable.

APPENDIX TABLE 9. PROTEIN CONTENT AND AMINO ACID COMPOSITION OF THE GRAIN OF PROGENY AND PARENTAL TYPES DESCRIBED IN APPENDIX TABLE 8[a]

Sample Code	DHP	175	25	26	27	28	29	30	31
Kjeldahl Protein, %[b]	16.59[e]	13.44	15.42	15.68	16.67	15.97	14.72	14.40	14.55
Total Amino Acids, %[c]	15.29	12.84	14.58	14.55	15.67	14.58	13.86	13.53	13.48
Nitrogen recovery, %[d]	92.14	95.50	94.55	92.80	93.99	91.30	94.16	93.95	92.64
Lysine	0.59	0.28	0.36	0.30	0.39	0.32	0.32	0.31	0.32
Histidine	0.32	0.31	0.38	0.35	0.36	0.36	0.35	0.32	0.36
Ammonia	0.51	0.54	0.62	0.61	0.70	0.61	0.60	0.54	0.61
Arginine	0.56	0.53	0.64	0.60	0.69	0.65	0.57	0.55	0.63
Aspartic Acid	1.16	0.71	0.80	0.82	0.86	0.80	0.76	0.78	0.69
Threonine	0.54	0.35	0.43	0.44	0.47	0.44	0.38	0.41	0.37
Serine	0.71	0.68	0.76	0.78	0.83	0.77	0.72	0.73	0.68
Glutamic Acid	3.80	3.93	4.44	4.57	4.87	4.48	4.29	4.21	4.11
Proline	1.68	1.40	1.56	1.60	1.68	1.57	1.49	1.41	1.46
Glycine	0.72	0.59	0.66	0.69	0.74	0.67	0.63	0.63	0.61
Alanine	0.69	0.50	0.51	0.59	0.59	0.53	0.54	0.52	0.49
Cystine	0.15	0.20	0.25	0.27	0.22	0.23	0.22	0.19	0.24
Valine	0.58	0.42	0.47	0.47	0.49	0.47	0.44	0.43	0.44
Methionine	0.34	0.21	0.24	0.22	0.24	0.23	0.22	0.21	0.21
Isoleucine	0.39	0.32	0.36	0.35	0.35	0.36	0.33	0.34	0.33
Leucine	1.00	0.86	0.96	0.99	1.04	0.97	0.92	0.90	0.89
Tyrosine	0.56	0.43	0.48	0.50	0.47	0.48	0.46	0.43	0.44
Phenylalanine	0.80	0.58	0.65	0.67	0.69	0.66	0.62	0.62	0.61

[a] Results are reported on an "as is" moisture basis, g. amino acid per 100 g. sample.

[b] N x 5.7

[c] The total amino acids as % of sample.

[d] Nitrogen recovery, g. amino acid nitrogen per 100 g. Kjeldahl nitrogen.

[e] N x 5.83

APPENDIX TABLE 9 (continued)

Sample Code	322	323	324	325	326	327	328	329	330	331
Kjeldahl Protein, %	15.38	15.25	15.89	16.38	16.50	15.10	14.49	14.92	15.86	15.86
Total Amino Acids, %	14.56	14.24	14.03	15.33	16.04	14.37	13.80	14.20	14.60	15.35
Nitrogen recovery, %	91.94	93.36	88.26	93.59	97.21	95.17	95.26	95.21	92.05	96.79
Lysine	0.25	0.31	0.33	0.36	0.38	0.31	0.31	0.34	0.33	0.36
Histidine	0.29	0.38	0.37	0.39	0.41	0.34	0.34	0.36	0.34	0.36
Ammonia	0.53	0.67	0.66	0.66	0.74	0.59	0.61	0.60	0.63	0.69
Arginine	0.50	0.68	0.60	0.65	0.72	0.58	0.60	0.63	0.61	0.75
Aspartic Acid	0.81	0.75	0.76	0.79	0.85	0.75	0.78	0.76	0.85	0.82
Threonine	0.44	0.40	0.37	0.41	0.46	0.43	0.43	0.41	0.44	0.44
Serine	0.80	0.74	0.76	0.78	0.84	0.77	0.72	0.74	0.80	0.81
Glutamic Acid	4.80	4.43	4.43	4.82	5.06	4.44	4.29	4.35	4.67	4.82
Proline	1.49	1.55	1.51	1.73	1.72	1.59	1.50	1.56	1.63	1.64
Glycine	0.69	0.64	0.63	0.68	0.71	0.66	0.61	0.64	0.67	0.70
Alanine	0.58	0.49	0.53	0.52	0.60	0.54	0.44	0.52	0.55	0.56
Cystine	0.20	0.23	0.18	0.22	0.22	0.22	0.22	0.25	0.28	0.24
Valine	0.47	0.44	0.40	0.48	0.49	0.46	0.44	0.45	0.50	0.47
Methionine	0.20	0.21	0.21	0.24	0.23	0.20	0.21	0.21	0.22	0.22
Isoleucine	0.35	0.33	0.31	0.38	0.38	0.35	0.33	0.35	0.36	0.36
Leucine	0.97	0.92	0.90	1.01	1.03	0.95	0.91	0.93	0.98	0.99
Tyrosine	0.51	0.45	0.45	0.51	0.51	0.47	0.45	0.48	0.47	0.44
Phenylalanine	0.66	0.62	0.62	0.70	0.72	0.71	0.61	0.63	0.69	0.68

APPENDIX TABLE 9 (continued)

Sample Code	332	333	334	335	336	337	338	339	340	341
Kjeldahl Protein, %	15.95	15.60	15.39	15.83	15.82	15.68	16.50	15.36	16.73	15.33
Total Amino Acids, %	14.22	14.74	13.75	14.06	14.06	14.29	15.05	13.86	14.74	14.54
Nitrogen recovery, %	89.16	94.48	89.31	88.79	88.90	91.12	91.23	90.23	88.09	94.84
Lysine	0.30	0.31	0.34	0.30	0.36	0.33	0.35	0.35	0.36	0.35
Histidine	0.35	0.37	0.33	0.34	0.35	0.31	0.31	0.30	0.36	0.36
Ammonia	0.66	0.64	0.65	0.62	0.67	0.69	0.71	0.66	0.73	0.67
Arginine	0.62	0.59	0.65	0.53	0.66	0.58	0.52	0.49	0.67	0.68
Aspartic Acid	0.80	0.80	0.76	0.81	0.76	0.79	0.82	0.83	0.82	0.81
Threonine	0.40	0.40	0.41	0.39	0.38	0.40	0.41	0.41	0.43	0.39
Serine	0.72	0.79	0.78	0.74	0.76	0.74	0.80	0.77	0.81	0.77
Glutamic Acid	4.43	4.66	4.00	4.50	4.39	4.55	4.88	4.29	4.31	4.50
Proline	1.52	1.64	1.51	1.53	1.50	1.53	1.58	1.48	1.64	1.58
Glycine	0.64	0.65	0.67	0.64	0.63	0.67	0.68	0.64	0.75	0.66
Alanine	0.55	0.52	0.51	0.55	0.51	0.55	0.56	0.53	0.55	0.54
Cystine	0.20	0.25	0.19	0.20	0.21	0.20	0.18	0.17	0.18	0.19
Valine	0.43	0.45	0.43	0.41	0.41	0.42	0.44	0.42	0.44	0.45
Methionine	0.22	0.23	0.27	0.22	0.22	0.24	0.30	0.24	0.23	0.21
Isoleucine	0.32	0.36	0.40	0.31	0.31	0.28	0.39	0.34	0.33	0.34
Leucine	0.94	0.94	0.92	0.90	0.90	0.92	0.97	0.89	0.99	0.94
Tyrosine	0.45	0.49	0.42	0.45	0.44	0.46	0.47	0.46	0.47	0.45
Phenylalanine	0.62	0.66	0.51	0.62	0.62	0.64	0.67	0.61	0.68	0.66

APPENDIX TABLE 9 (continued)

Sample Code	342	343	344	345	346	347	348	349	350	351
Kjeldahl Protein, %	16.15	16.01	15.92	15.33	15.42	16.00	15.22	15.32	15.51	15.48
Total Amino Acids, %	14.97	14.24	13.91	14.56	14.93	14.77	14.74	15.09	13.98	14.89
Nitrogen recovery, %	92.71	88.92	87.36	94.98	96.79	92.32	96.87	98.47	90.14	96.19
Lysine	0.35	0.35	0.33	0.36	0.36	0.30	0.35	0.32	0.34	0.32
Histidine	0.33	0.33	0.31	0.39	0.37	0.35	0.37	0.37	0.35	0.34
Ammonia	0.70	0.70	0.67	0.69	0.64	0.70	0.61	0.63	0.66	0.69
Arginine	0.64	0.63	0.62	0.70	0.63	0.52	0.61	0.61	0.63	0.66
Aspartic Acid	0.82	0.83	0.79	0.79	0.80	0.78	0.81	0.82	0.77	0.82
Threonine	0.40	0.40	0.46	0.41	0.42	0.42	0.42	0.41	0.42	0.43
Serine	0.79	0.77	0.76	0.73	0.76	0.83	0.73	0.79	0.76	0.79
Glutamic Acid	4.75	4.26	4.00	4.46	4.60	4.83	4.60	4.75	4.40	4.63
Proline	1.57	1.57	1.57	1.60	1.74	1.57	1.61	1.65	1.51	1.63
Glycine	0.70	0.67	0.68	0.65	0.64	0.69	0.66	0.67	0.62	0.68
Alanine	0.56	0.53	0.54	0.50	0.50	0.54	0.59	0.61	0.52	0.56
Cystine	0.19	0.18	0.22	0.23	0.26	0.17	0.25	0.26	0.20	0.19
Valine	0.43	0.42	0.41	0.45	0.47	0.43	0.46	0.47	0.41	0.47
Methionine	0.27	0.22	0.22	0.22	0.23	0.17	0.23	0.23	0.21	0.21
Isoleucine	0.41	0.32	0.31	0.35	0.37	0.33	0.36	0.37	0.31	0.35
Leucine	0.95	0.94	0.92	0.94	0.96	0.95	0.96	0.98	0.89	0.97
Tyrosine	0.46	0.49	0.47	0.48	0.49	0.50	0.48	0.49	0.44	0.48
Phenylalanine	0.64	0.62	0.63	0.63	0.67	0.67	0.65	0.66	0.54	0.67

APPENDIX TABLE 9 (concluded).

Sample Code	352	353	354	355	356	357	358	359	360	361	362
Kjeldahl Prot., %	14.81	15.22	15.48	15.27	15.27	15.68	16.00	16.09	16.21	15.54	15.36
Tot. Amino Acids %	13.75	13.65	13.76	14.87	14.10	14.09	14.61	14.73	14.97	14.68	14.35
N recovery, %	92.82	89.66	88.86	97.35	92.35	89.86	91.31	91.55	92.35	94.47	93.40
Lysine	0.36	0.24	0.33	0.35	0.32	0.34	0.29	0.34	0.36	0.35	0.28
Histidine	0.36	0.21	0.30	0.35	0.31	0.33	0.36	0.31	0.33	0.34	0.31
Ammonia	0.63	0.49	0.64	0.65	0.59	0.68	0.67	0.69	0.68	0.63	0.61
Arginine	0.65	0.37	0.50	0.65	0.53	0.63	0.67	0.57	0.67	0.62	0.59
Aspartic Acid	0.76	0.83	0.73	0.83	0.79	0.81	0.81	0.81	0.86	0.84	0.82
Threonine	0.39	0.44	0.40	0.44	0.43	0.41	0.39	0.41	0.42	0.44	0.43
Serine	0.69	0.78	0.73	0.77	0.75	0.78	0.71	0.81	0.79	0.77	0.76
Glutamic Acid	4.16	4.42	4.42	4.60	4.41	4.12	4.54	4.67	4.67	4.54	4.45
Proline	1.49	1.43	1.53	1.60	1.55	1.55	1.61	1.56	1.54	1.55	1.56
Glycine	0.60	0.67	0.63	0.67	0.64	0.65	0.67	0.66	0.68	0.66	0.65
Alanine	0.47	0.55	0.57	0.57	0.49	0.52	0.56	0.52	0.54	0.58	0.57
Cystine	0.24	0.18	0.14	0.21	0.24	0.21	0.21	0.17	0.17	0.22	0.23
Valine	0.43	0.44	0.40	0.45	0.44	0.44	0.45	0.44	0.44	0.46	0.46
Methionine	0.21	0.21	0.21	0.21	0.28	0.28	0.22	0.32	0.31	0.23	0.22
Isoleucine	0.34	0.33	0.29	0.35	0.33	0.38	0.34	0.39	0.42	0.36	0.36
Leucine	0.89	0.94	0.88	0.95	0.92	0.94	0.96	0.94	0.96	0.95	0.95
Tyrosine	0.46	0.47	0.45	0.50	0.46	0.45	0.49	0.47	0.48	0.49	0.47
Phenylalanine	0.61	0.66	0.60	0.72	0.63	0.55	0.66	0.65	0.66	0.64	0.65

APPENDIX TABLE 10. PROTEIN CONTENT AND AMINO ACID COMPOSITION OF THE PROTEIN (N x 5.7) OF PROGENY AND PARENTAL TYPES DESCRIBED IN APPENDIX TABLE 8[a]

Sample Code	DHP	175	25	26	27	28	29	30	31
Lysine	3.90	2.18	2.47	2.04	2.51	2.20	2.33	2.29	2.39
Histidine	2.14	2.44	2.61	2.35	2.29	2.45	2.54	2.33	2.68
Ammonia	3.41	4.18	4.23	4.13	4.47	4.20	4.35	4.01	4.54
Arginine	3.72	4.13	4.36	4.07	4.37	4.47	4.13	4.08	4.65
Aspartic Acid	7.69	5.52	5.48	5.49	5.46	5.46	5.51	5.79	5.12
Threonine	3.58	2.75	2.98	2.99	3.00	2.98	2.75	3.05	2.73
Serine	4.68	5.30	5.20	5.28	5.31	5.27	5.19	5.39	5.05
Glutamic Acid	25.17	30.61	30.47	30.78	31.07	30.67	30.96	31.12	30.45
Proline	11.13	10.87	10.74	10.78	10.70	10.72	10.78	10.42	10.81
Glycine	4.75	4.57	4.52	4.61	4.69	4.60	4.52	4.65	4.50
Alanine	4.56	3.88	3.53	3.95	3.75	3.61	3.88	3.84	3.63
Cystine	1.02	1.59	1.74	1.83	1.39	1.57	1.46	1.37	1.76
Valine	3.86	3.29	3.20	3.14	3.15	3.22	3.18	3.16	3.26
Methionine	2.27	1.62	1.67	1.50	1.51	1.59	1.56	1.57	1.57
Isoleucine	2.60	2.47	2.47	2.38	2.24	2.45	2.41	2.51	2.44
Leucine	6.64	6.67	6.61	6.63	6.61	6.62	6.63	6.64	6.57
Tyrosine	3.68	3.31	3.31	3.36	2.99	3.25	3.29	3.18	3.24
Phenylalanine	5.30	4.54	4.47	4.52	4.40	4.51	4.45	4.59	4.50

[a] Results are reported on an "as is" moisture basis, g. amino acid per 100 g. protein (N x 5.7) corrected to 100% recovery of Kjeldahl protein.

APPENDIX TABLE 10 (continued)

Sample Code	322	323	324	325	326	327	328	329	330	331
Lysine	1.71	2.16	2.35	2.36	2.37	2.15	2.24	2.41	2.22	2.34
Histidine	1.98	2.64	2.67	2.57	2.54	2.37	2.46	2.55	2.24	2.35
Ammonia	3.68	4.71	4.71	4.39	4.59	4.08	4.41	4.23	4.20	4.51
Arginine	3.46	4.80	4.27	4.33	4.46	4.05	4.37	4.44	4.02	4.92
Aspartic Acid	5.55	5.25	5.40	5.23	5.28	5.22	5.67	5.32	5.66	5.33
Threonine	3.02	2.83	2.65	2.72	2.85	3.00	3.08	2.89	2.93	2.87
Serine	5.49	5.18	5.38	5.16	5.22	5.40	5.23	5.20	5.29	5.26
Glutamic Acid	33.01	31.14	31.58	31.91	31.47	30.95	31.12	30.62	31.09	31.37
Proline	10.28	10.88	10.80	11.46	10.68	11.05	10.85	10.97	10.87	10.70
Glycine	4.75	4.49	4.48	4.51	4.43	4.61	4.44	4.52	4.49	4.53
Alanine	4.00	3.46	3.80	3.45	3.74	3.73	3.20	3.63	3.66	3.63
Cystine	1.40	1.61	1.28	1.44	1.37	1.56	1.60	1.75	1.85	1.54
Valine	3.20	3.07	2.84	3.19	3.02	3.23	3.16	3.15	3.33	3.09
Methionine	1.41	1.45	1.52	1.56	1.44	1.41	1.54	1.50	1.43	1.40
Isoleucine	2.42	2.36	2.19	2.50	2.36	2.42	2.39	2.45	2.38	2.37
Leucine	6.71	6.47	6.44	6.68	6.38	6.64	6.58	6.55	6.51	6.42
Tyrosine	3.50	3.13	3.21	3.37	3.16	3.30	3.29	3.38	3.12	2.90
Phenylalanine	4.56	4.36	4.44	4.63	4.48	4.92	4.39	4.44	4.59	4.42

APPENDIX TABLE 10 (continued)

Sample Code	332	333	334	335	336	337	338	339	340	341
Lysine	2.11	2.07	2.46	2.16	2.52	2.34	2.32	2.50	2.41	2.38
Histidine	2.50	2.50	2.38	2.42	2.48	2.13	2.05	2.14	2.43	2.44
Ammonia	4.66	4.37	4.74	4.43	4.73	4.86	4.75	4.76	4.92	4.59
Arginine	3.96	3.97	4.70	3.77	4.65	4.03	3.47	3.51	4.92	4.65
Aspartic Acid	5.67	5.45	5.54	5.75	5.41	5.50	5.43	5.94	5.56	5.56
Threonine	2.83	2.71	2.99	2.76	2.67	2.82	2.77	2.93	2.95	2.68
Serine	5.09	5.35	5.66	5.30	5.41	5.20	5.33	5.55	5.51	5.27
Glutamic Acid	31.24	31.59	29.08	32.09	31.17	31.81	32.45	30.87	29.24	30.95
Proline	10.73	11.08	11.01	10.88	10.67	10.71	10.53	10.66	11.09	10.84
Glycine	4.55	4.38	4.85	4.58	4.44	4.70	4.50	4.60	5.08	4.53
Alanine	3.85	3.50	3.69	3.92	3.60	3.83	3.69	3.80	3.70	3.73
Cystine	1.80	1.69	1.35	1.44	1.51	1.39	1.18	1.23	1.23	1.29
Valine	3.02	3.09	3.14	2.95	2.92	2.95	2.94	3.03	2.97	3.12
Methionine	1.58	1.55	1.99	1.55	1.54	1.65	2.01	1.72	1.56	1.46
Isoleucine	2.27	2.42	2.91	2.21	2.19	1.96	2.61	2.48	2.21	2.35
Leucine	6.62	6.39	6.73	6.45	6.42	6.42	6.42	6.43	6.69	6.46
Tyrosine	3.15	3.34	3.05	3.21	3.12	3.22	3.16	3.29	3.22	3.12
Phenylalanine	4.37	4.51	3.68	4.42	4.42	4.44	4.46	4.40	4.58	4.52

APPENDIX TABLE 10 (continued)

Sample Code	342	343	344	345	346	347	348	349	350	351
Lysine	2.36	2.50	2.29	2.49	2.39	2.03	2.39	2.12	2.41	2.17
Histidine	2.20	2.35	2.17	2.68	2.49	2.40	2.52	2.43	2.49	2.31
Ammonia	4.70	4.88	4.72	4.71	4.31	4.73	4.14	4.20	4.74	4.63
Arginine	4.31	4.40	4.23	4.76	4.22	3.54	4.11	4.01	4.53	4.41
Aspartic Acid	5.51	5.84	5.54	5.42	5.36	5.32	5.47	5.45	5.51	5.49
Threonine	2.69	2.82	2.23	2.79	2.82	2.87	2.83	2.70	3.00	2.91
Serine	5.27	5.41	5.37	5.00	5.13	5.67	4.95	5.22	5.45	5.28
Glutamic Acid	31.77	29.93	28.12	30.61	30.86	32.80	31.16	31.47	31.49	31.06
Proline	10.49	11.05	11.03	10.98	11.69	10.65	10.91	10.96	10.78	10.94
Glycine	4.67	4.74	4.78	4.43	4.31	4.68	4.48	4.45	4.42	4.53
Alanine	3.73	3.73	3.81	3.39	3.38	3.65	4.00	4.05	3.71	3.74
Cystine	1.28	1.28	1.54	1.54	1.75	1.14	1.67	1.70	1.44	1.30
Valine	2.89	2.94	2.90	3.08	3.15	2.89	3.12	3.14	2.94	3.17
Methionine	1.82	1.58	1.54	1.51	1.52	1.17	1.57	1.52	1.49	1.44
Isoleucine	2.73	2.22	2.14	2.43	2.47	2.21	2.45	2.47	2.21	2.37
Leucine	6.34	6.59	6.45	6.42	6.46	6.45	6.53	6.51	6.33	6.50
Tyrosine	3.06	3.43	3.32	3.32	3.29	3.40	3.25	3.24	3.13	3.24
Phenylalanine	4.29	4.39	4.46	4.29	4.52	4.56	4.40	4.36	3.86	4.50

APPENDIX TABLE 10 (concluded)

Sample Code	352	353	354	355	356	357	358	359	360	361	362
Lysine	2.59	2.73	2.41	2.34	2.16	2.44	2.01	2.31	2.38	2.41	1.93
Histidine	2.59	1.53	2.18	2.38	2.22	2.33	2.45	2.11	2.22	2.33	2.17
Ammonia	4.62	3.56	4.67	4.37	4.17	4.85	4.60	4.66	4.54	4.31	4.22
Arginine	4.72	2.67	3.67	4.36	3.77	4.48	4.55	3.84	4.45	4.24	4.12
Aspartic Acid	5.54	6.07	5.33	5.58	5.60	5.76	5.55	5.50	5.71	5.72	5.71
Threonine	2.85	3.19	2.89	2.94	3.06	2.93	2.68	2.80	2.80	2.99	2.97
Serine	5.03	5.70	5.28	5.15	5.34	5.56	4.88	5.47	5.28	5.28	5.27
Glutamic Acid	30.25	32.36	32.11	30.95	31.28	29.30	31.10	31.71	31.18	30.96	30.95
Proline	10.84	10.46	11.12	10.75	10.98	11.05	11.01	10.60	10.31	10.54	10.83
Glycine	4.35	4.92	4.60	4.51	4.54	4.62	4.61	4.45	4.51	4.52	4.53
Alanine	3.36	3.99	4.12	3.86	3.46	3.74	3.85	3.55	3.59	3.94	3.98
Cystine	1.78	1.33	1.03	1.40	1.72	1.46	1.41	1.12	1.16	1.54	1.58
Valine	3.14	3.22	2.88	3.01	3.08	3.13	3.11	2.98	2.95	3.13	3.28
Methionine	1.51	1.56	1.53	1.43	1.98	2.02	1.50	2.19	2.06	1.55	1.50
Isoleucine	2.51	2.45	2.14	2.34	2.36	2.73	2.32	2.68	2.78	2.43	2.50
Leucine	6.49	6.87	6.39	6.42	6.51	6.67	6.55	6.41	6.38	6.49	6.58
Tyrosine	3.35	3.44	3.25	3.36	3.26	3.23	3.34	3.21	3.20	3.35	3.29
Phenylalanine	4.45	4.83	4.38	4.82	4.43	3.89	4.49	4.38	4.41	4.36	4.55

APPENDIX TABLE 11. MEAN AND STANDARD DEVIATIONS OF CONTROL TREATMENTS AND POTENTIAL HYBRIDS PRESENTED AS % OF SAMPLE (DATA FROM APPENDIX TABLE 9)

	W/EACA		W/O EACA		Potential Hybrids	
	$\bar{X}$	S	$\bar{X}$	S	$\bar{X}$	S
Amino Acid Protein	14.92	0.52	13.63	0.20	14.48	0.52
Kjeldahl Protein	15.94	0.54	14.56	0.16	15.65	0.48
Nitrogen Recovery	93.16	1.44	93.58	0.82	92.53	3.02
Lysine	0.34	0.04	0.32	0.01	0.33	0.03
Histidine	0.36	0.01	0.34	0.02	0.34	0.03
Ammonia	0.64	0.04	0.58	0.04	0.65	0.05
Arginine	0.65	0.04	0.74	0.05	0.61	0.07
Aspartic Acid	0.82	0.03	0.78	0.02	0.80	0.03
Threonine	0.45	0.02	0.39	0.02	0.77	0.03
Serine	0.79	0.03	0.71	0.03	0.77	0.03
Glutamic Acid	4.59	0.19	4.20	0.09	4.51	0.23
Proline	1.60	0.05	1.45	0.04	1.57	0.07
Glycine	0.69	0.04	0.62	0.01	0.66	0.03
Alanine	0.56	0.04	0.52	0.03	0.54	0.03
Cystine	0.24	0.02	0.22	0.03	0.21	0.03
Valine	0.48	0.01	0.44	0.01	0.44	0.02
Methionine	0.23	0.01	0.21	0.01	0.23	0.03
Isoleucine	0.36	0.01	0.33	0.01	0.35	0.03
Leucine	0.99	0.04	0.90	0.02	0.94	0.03
Tyrosine	0.48	0.01	0.44	0.02	0.47	0.02
Phenylalanine	0.67	0.02	0.62	0.01	0.64	0.04

APPENDIX TABLE 12. MEAN AND STANDARD DEVIATIONS FOR CONTROL TREATMENTS AND POTENTIAL HYBRIDS PRESENTED AS % OF KJELDAHL PROTEIN CORRECTED TO 100% RECOVERY (DATA FROM APPENDIX TABLE 10)

	W/EACA		W/O EACA		Potential Hybrids	
	$\bar{X}$	S	$\bar{X}$	S	$\bar{X}$	S
Lysine	2.31	0.22	2.34	0.05	2.30	0.19
Histidine	2.43	0.14	2.52	0.18	2.35	0.22
Ammonia	4.26	0.15	4.30	0.27	4.50	0.30
Arginine	4.32	0.17	4.29	0.32	4.20	0.47
Aspartic Acid	5.47	0.02	5.49	0.34	5.53	0.19
Threonine	2.99	0.01	2.84	0.18	2.80	0.36
Serine	5.27	0.05	5.21	0.17	5.30	0.19
Glutamic Acid	30.75	0.25	30.85	0.35	31.13	0.97
Proline	10.74	0.03	10.67	0.22	10.85	0.27
Glycine	4.61	0.07	4.56	0.08	4.56	0.15
Alanine	3.78	0.18	3.71	0.13	3.71	0.21
Cystine	1.53	0.19	1.63	0.20	1.46	0.21
Valine	3.18	0.04	3.20	0.05	3.06	0.12
Methionine	1.57	0.08	1.57	0.01	1.59	0.21
Isoleucine	2.45	0.10	2.39	0.05	2.40	0.19
Leucine	6.62	0.01	6.61	0.04	6.51	0.12
Tyrosine	3.24	0.16	3.23	0.06	3.25	0.12
Phenylalanine	4.48	0.05	4.51	0.07	4.45	0.21

APPENDIX TABLE 13. t TEST FOR 2 MEANS (H_o: $\mu_1 = \mu_2$)[a,b] (DATA FROM APPENDIX TABLES 9 AND 10)

	As % of Sample				100% Recovery Kjeldahl Protein			
	W/EACA vs W/O EACA ($t_{0.05,5} = 2.571$)		W/EACA vs Potential Hybrids ($t_{0.05,43} = 2.017$)		W/EACA vs W/O EACA ($t_{0.05,5} = 2.571$)		W/EACA vs Potential Hybrids ($t_{0.05,43} = 2.017$)	
	t	Accept H_o	t	Accept H_o	t	Accept H_o	t	Accept H_o
Amino Acid Protein	3.99	No	1.58	Yes				
Kjeldahl Protein	4.20	No	1.11	Yes				
Nitrogen Recovery	0.45	Yes	0.40	Yes				
Lysine	1.08	Yes	0.56	Yes	0.24	Yes	0.10	Yes
Histidine	1.53	Yes	1.25	Yes	0.77	Yes	0.70	Yes
Ammonia	1.63	Yes	0.38	Yes	0.27	Yes	1.55	Yes
Arginine	2.08	Yes	1.09	Yes	0.17	Yes	0.50	Yes
Aspartic Acid	2.71	No	1.20	Yes	0.01	Yes	0.62	Yes
Threonine	4.06	No	2.56	No	1.66	Yes	1.03	Yes
Serine	3.35	No	1.20	Yes	0.63	Yes	0.31	Yes
Glutamic Acid	3.14	No	0.66	Yes	0.43	Yes	0.76	Yes
Proline	3.96	No	0.82	Yes	0.61	Yes	0.80	Yes
Glycine	3.06	No	1.68	Yes	0.85	Yes	0.65	Yes
Alanine	1.41	Yes	1.12	Yes	0.58	Yes	0.63	Yes
Cystine	1.44	Yes	1.84	Yes	0.68	Yes	0.63	Yes
Valine	5.86	No	3.51	No	0.66	Yes	1.94	Yes
Methionine	3.04	No	0.00	Yes	0.02	Yes	0.18	Yes
Isoleucine	4.91	No	0.62	Yes	1.03	Yes	0.51	Yes
Leucine	3.88	No	2.21	No	0.22	Yes	1.78	Yes
Tyrosine	3.74	No	0.88	Yes	0.09	Yes	0.15	Yes
Phenylalanine	4.85	No	1.40	Yes	0.81	Yes	0.28	Yes

[a] Zar, J. H. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, N. J. p. 105-107 (1974).

[b] Fryer, H. C. Concepts and Methods of Experimental Statistics. Allyn and Bacon, Inc., Boston, p. 175-178 (1966).

APPENDIX TABLE 14. t TEST FOR A MEAN EQUAL TO SOME CONSTANT (H_o: $\mu = c$) USING DATA FOR % OF SAMPLE[a] (DATA FROM APPENDIX TABLE 9)

	Control Constant Sample 175	W/EACA ($t_{0.05,3} = 3.182$)		W/O EACA ($t_{0.05,2} = 4.303$)		Potential Hybrids ($t_{0.05,40} = 2.021$)	
		t	Accept H_o	t	Accept H_o	t	Accept H_o
Amino Acid Protein	12.84	8.00	No	25.98	No	20.19	No
Kjeldahl Protein	13.44	9.26	No	32.48	No	29.48	No
Nitrogen Recovery	95.50	3.25	No	6.34	No	6.32	No
Lysine	0.28	3.00	Yes	8.66	No	10.67	No
Histidine	0.31	10.00	No	2.60	Yes	6.40	No
Ammonia	0.54	5.00	No	1.73	Yes	14.09	No
Arginine	0.53	6.00	No	7.21	No	7.32	No
Aspartic Acid	0.71	7.33	No	27.71	No	19.21	No
Threonine	0.35	10.00	No	3.46	Yes	22.41	No
Serine	0.68	7.33	No	1.73	Yes	19.21	No
Glutamic Acid	3.93	6.95	No	5.20	No	16.15	No
Proline	1.40	8.00	No	2.17	Yes	15.55	No
Glycine	0.59	5.00	No	5.20	No	14.94	No
Alanine	0.50	3.00	Yes	1.15	Yes	8.54	No
Cystine	0.20	4.00	No	1.15	Yes	2.13	No
Valine	0.42	12.00	No	3.46	Yes	6.40	No
Methionine	0.21	4.00	No	0.00	Yes	4.27	No
Isoleucine	0.32	8.00	No	1.73	Yes	6.40	No
Leucine	0.86	6.50	No	3.46	Yes	17.07	No
Tyrosine	0.43	10.00	No	0.87	Yes	12.81	No
Phenylalanine	0.58	9.00	No	6.93	No	9.60	No

[a] Zar, J. H. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, N. J. p. 89 (1974).

APPENDIX TABLE 15. t TEST FOR A MEAN EQUAL TO SOME CONSTANT (H_o: $\mu = c$) USING DATA FOR 100% RECOVERY OF KJELDAHL PROTEIN[a] (DATA FROM APPENDIX TABLE 10)

	Control Constant Sample 175	W/EACA ($t_{0.05,3} = 3.182$)		W/O EACA ($t_{0.05,2} = 4.303$)		Potential Hybrids ($t_{0.05,40} = 2.021$)	
		t	Accept H_o	t	Accept H_o	t	Accept H_o
Lysine	2.18	1.18	Yes	5.54	No	4.04	No
Histidine	2.44	0.14	Yes	0.77	Yes	2.62	No
Ammonia	4.18	1.07	Yes	0.77	Yes	8.11	No
Arginine	4.13	2.24	Yes	0.87	Yes	0.95	Yes
Aspartic Acid	5.52	5.00	No	0.15	Yes	0.34	Yes
Threonine	2.75	48.00	No	0.87	Yes	0.89	Yes
Serine	5.30	1.20	Yes	0.92	Yes	0.00	Yes
Glutamic Acid	30.61	1.12	Yes	1.14	Yes	3.43	No
Proline	10.87	8.67	No	1.57	Yes	0.47	No
Glycine	4.57	1.14	Yes	0.22	Yes	0.43	Yes
Alanine	3.88	1.11	Yes	2.26	Yes	5.18	No
Cystine	1.59	0.63	Yes	0.35	Yes	3.96	No
Valine	3.29	5.50	No	3.12	Yes	12.27	No
Methionine	1.62	1.25	Yes	8.66	No	0.91	Yes
Isoleucine	2.47	0.40	Yes	2.77	Yes	2.36	No
Leucine	6.67	10.00	No	2.60	Yes	8.54	No
Tyrosine	3.31	0.88	Yes	2.31	Yes	3.20	No
Phenylalanine	4.54	2.40	Yes	0.74	Yes	0.61	Yes

[a] Zar, J. H. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, N. J. p. 89 (1974).

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PROTEIN AND STRUCTURAL ANALYSIS OF POTENTIAL WIDE-HYBRID CEREALS

by

GENE HARVEY KELTNER

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Hybrid research in cereals to increase the genetic variability is making a significant contribution toward improving the food resources of the world. It is improving the yield, adaptability, and nutritional quality of cereal crops. The research presented in this thesis describes studies toward detecting successful wide-hybrid crosses between wheat, rye, and barley.

Part I describes a cereal kernel sectioning technique developed for observing structural characteristics of parental and hybrid kernels. The method used aqueous dimethyl sulfoxide (DMSO) to soften the kernel held in a cork matrix. After treatment the kernels were sectioned on a rotary microtome equipped with a razor blade adapter. Aleurone cells, endosperm cells, and starch granules were studied under polarized light and Nomarski interference-contrast microscopy. The procedure is less laborious than other preparatory techniques and it maintains the integrity of the kernel structure.

Part II describes protein analysis of the wide-hybrids generated. Total protein, amino acid analysis, and electrophoretic studies were conducted to detect inherited and altered protein changes in the progeny of the wide-hybrid crosses.